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UNIVERSITY OF ALBERTA

CALCIUM CHANNEL BLOCKERS AND INTESTINAL NUTRIENT UPTAKE

BY

© Dianne A. Hyson

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH IN

PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

IN

EXPERIMENTAL MEDICINE

DEPARTMENT OF MEDICINE

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DEDICATION

To my husband, Arne

for his love, friendship and support in all areas of my life.

To my parents

who have enriched my life by their example, encouragement and love.

To my sister, Susan

my closest friend and confidante.

ABSTRACT

Calcium channel blockers (CCBs) are widely used in clinical practice but there is only limited information of their effect on intestinal function. Thus, a study was designed to test the hypotheses that a) there are multiple calcium (Ca) channels in rabbit jejunum and b) that these Ca channels influence active and/or passive uptake of lipids and hexoses in the jejunum. A study was undertaken to determine the effects of a dihydropyridine, nisoldipine (N) or a phenylalkylamine, verapamil (V) on the jejunal uptake of lipids and hexoses. In addition, the effects of these drugs on ionic fluxes in the jejunum was investigated.

The uptake of cholesterol, long chain fatty acids, D-glucose, galactose and L-glucose was studied using a previously validated in vitro technique which involved determining the incorporation of ^{14}C-labelled substrates into jejunal mucosa. The ionic fluxes were studied using Ussing chamber experiments to determine the effects of CCBs on short circuit current (Isc).

Experiments were designed to determine the effects of short term or "acute" CCB exposure to intact isolated jejunal segments. In these experiments male New Zealand white rabbit jejunum was mucosally exposed to a dose range (10^{-8}-10^{-4} M) of N or V for 6 and 36 minutes. The animals were fed either a low cholesterol diet (LCD) or high cholesterol diet (2.8%, HCD). Acute exposure to N or V did not affect the jejunal uptake of cholesterol, palmitic acid, or D-glucose in LCD or HCD.

In a second series of experiments the animals were fed "chronic" (3 week feeding) doses of N (1 mg/kg body weight) or V (4 mg/kg body weight). The chronic feeding of N or V had a variable effect on lipid uptake depending upon the cholesterol content of the diet: adding N or V to LCD increased cholesterol uptake, N enhanced cholesterol uptake in HCD yet V lowered uptake in HCD. Both N and V increased the uptake of stearic acid in LCD,

N had no effect on fatty acid uptake in HCD whereas V lowered the uptake of stearic and linoleic acids.

Chronic administration of N or V had differential effects on apparent kinetic parameters of glucose and galactose uptake; these effects were also influenced by the cholesterol content of the diet. The maximal transport capacities of both glucose and galactose were greater in LCD plus N and in HCD plus N as compared to LCD or HCD without N. There was no effect of N on the apparent affinity constant (Km^*) for glucose but there was an increased Km^* for galactose in both LCD and HCD. The maximal transport capacity (Jmax) for glucose decreased in LCD plus V but increased in HCD plus V; galactose was affected in an opposite manner with Jmax being increased in LCD plus V but decreased in HCD plus V. V feeding decreased the Km^* for both glucose and galactose in LCD but increased the Km^*'s in HCD. Passive uptake of L-glucose was decreased in LCD plus N and increased in LCD plus V, but was unchanged in HCD plus N or HCD plus V as compared with HCD alone.

In a final series of studies isolated jejunal segments were serosally exposed to N, V, or N plus V in LCD or HCD. There was no dose-dependent inhibition of basal jejunal Isc by the serosal addition of either CCB or the combination of both agents in animals fed LCD or HCD. However, both N and V reduced the magnitude of the stimulated Isc response to glucose or theophylline in LCD but not HCD.

The alterations in jejunal nutrient uptake and Isc responses were not due to variation in the animals' food intake, body weight gain, mucosal surface area or membrane potential. Thus, it is concluded that the chronic administration of N or V results in an intestinal adaptive process that alters the jejunal uptake of lipids and hexoses, the direction of which is influenced by the class of drug and the cholesterol content of the diet.

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LIST OF ABBREVIATIONS

ACAT	Acyl-Coenzyme A: cholesterol acyltransferase
apo-AI	Apoprotein AI
apo-B	Apoprotein B
apo-E	Apoprotein E
ATP	Adenosine triphosphate
BBM	Brush border membrane
BLM	Basolateral membrane
Ca	Calcium
CAM	Calmodulin
cAMP	Cyclic adenosine monophosphate
CCB	Calcium channel blocker
CDPC	Calcium dependent potassium channel
CE	Cholesterol esters
Cl	Chloride
CM	Chylomicrons
DFMO	DL-α-difluoromethylornithine
DG	Diacylglycerol
EGTA	Ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N', -tetracetic acid
ER	Endoplasmic reticulum
FABP	Fatty acid binding protein
HDL	High density lipoproteins
HMG-CoA reductase	3-hydroxy-3-methylglutaryl Coenzyme A reductase

IDL	Intermediate density lipoproteins
$Ins(1,4,5)P_3$	Inositol 1,4,5-triphosphate
K	Potassium
Km	Affinity constant
Km^*	Apparent affinity constant
Jmax	Maximal transport capacity
LaCl	Lanthanum chloride
LCAT	Lecithin-cholesterol acyltransferase
LDL	Low density lipoproteins
MFABP	Membrane fatty acid binding protein
N	Nisoldipine
Na	Sodium
ODC	Ornithine decarboxylase
Pd^*	Apparent passive permeability coefficient
$Pdt\text{-}Ins(4,5)P_2$	Phosphatidyl-inositol 4,5-biphosphate
PL	Phospholipids
ROC	Receptor-operated channels
ROCC	Receptor-operated calcium channels
TDC	Taurodeoxycholic acid
TG	Triglyceride
UWL	Unstirred water layer
V	Verapamil
VDCC	Voltage-dependent calcium channels
VLDL	Very low density lipoproteins

CHAPTER 1

INTRODUCTION

1.1) BACKGROUND AND AIMS OF RESEARCH

The antiatherosclerotic effect of calcium channel blockers (CCBs) has been reported in several animal studies (see Chapter 2 - Introduction) and has recently been suggested in human subjects (Lichtlen et al, 1990). In our experimental work with a cholesterol-fed rabbit model of atherosclerosis, we have observed that feeding the CCB, nisoldipine (N), concurrently with cholesterol reduces the accumulation of cholesterol in aortic tissue compared to cholesterol-fed controls (Senaratne et al, 1991). In addition, serum lipid levels of total cholesterol are reduced by approximately 40% in N fed animals versus controls on a high cholesterol diet. A similar reduction in serum cholesterol during the early phase of cholesterol and N feeding has been observed by others (Fronek, 1988). The mechanism for the antiatherosclerotic effect of CCBs and the reduced serum cholesterol levels in this and other animal models is unknown (see Chapter 2 - Introduction). It is reasonable to propose that one or more of the following hypotheses might apply:

i) CCBs influence the form and function of the intestine thus modifying intestinal uptake of lipids.

ii) CCBs influence enterocyte lipid metabolism and exit of lipoproteins.

iii) CCBs alter hepatic/extrahepatic metabolism of lipids.

iv) Macrophage function is influenced by feeding CCBs.

v) Biliary secretion of lipids is modified by CCBs.

The focus of the present experimental work was to test the first hypothesis, i.e. that CCBs modify intestinal uptake of lipids.

It was recently reported that rabbit intestine may have at least two voltage-dependent channels or binding sites that may be modulated differently by acute exposure to various classes of CCBs when added to the serosal surface (Homaidan et al, 1989). In addition, a role for calcium (Ca) and an influence of CCBs on the movement of intestinal electrolytes (sodium [Na], chloride [Cl] and potassium [K]) has been suggested (Donowitz, 1983; Donowitz and Asarkof, 1982; Hubel and Callanan, 1980). The effects of these agents on Na transport and permeability may have important implications in several passive and active nutrient uptake processes in the small intestine (see section 1.10.4 - 1.10.6). In addition, Ca is involved in numerous regulatory events in other tissues (see section 1.7.3, 1.8.1, 1.8.2). Thus, it is reasonable to speculate that CCBs, particularly when administered chronically, may alter intestinal transport function of several nutrients in addition to lipids.

Thus, a research project was designed with the following specific aims:

A) To test the hypothesis that there are multiple Ca channels in the jejunum of rabbits.

B) To determine if Ca channels play a role in active or passive uptake of hexoses and lipids in the jejunum.

1.2) GENERAL INTRODUCTION

The transport of water, nutrients and electrolytes is a vital function of the small intestine. The absorptive and secretory functions are carried out primarily by the columnar absorbing cells or enterocytes lining the lumen of the gastrointestinal tract. Thus, nutrient transport processes are dependent upon the properties of the brush border as well as the

basolateral membranes (BLM) of these cells. In addition, the state of the junctional complexes between adjacent cells and the state of the lateral intracellular spaces determines the general ionic permeabilities of the intestine and therefore has an important influence on absorptive and secretory events (Armstrong, 1987; Pappenheimer, 1990).

In addition to considering the epithelial barrier itself, the movement of solutes to the luminal interface is determined by the nature of an unstirred water layer (UWL) adjacent to the cells (Thomson, 1984; Thomson and Dietschy, 1984). Thus, studies of nutrient transport need to consider the movement of the solute through the UWL and the cell membranes in series. The importance and involvement of each parameter becomes apparent in the following discussion.

The absorptive function of the small intestine is a dynamic process that may continually adapt to changes in intraluminal content or physiologic and pathophysiological influences. As described in the ensuing discussion, modification of the properties of intestinal parameters including the absorptive cells, the tight junctions and the UWL may be important in mediating this adaptive process. Early research has focused on descriptive detail of alterations in nutrient transport and intestinal adaptation. Recently, work has turned towards elucidating the potential signals and the mechanisms for the observed changes.

As described herein, Ca is important as an almost universal intracellular signal in numerous cellular events. These include secretory, metabolic and transport processes as well as involvement in the growth of cells (Rasmussen, 1986a). A role for intracellular Ca in intestinal electrolyte transport has been established (Donowitz, 1983). The involvement of Ca in nutrient transport and intestinal adaptation is less clear. However, in view of the ubiquitous nature of this ion and its importance in cellular processes and proliferation, it is not unlikely that Ca plays a role in mediating the adaptive functions of the intestine.

1.3) INTESTINAL UPTAKE OF NUTRIENTS

1.3.1) GENERAL

In general, intestinal uptake of nutrients may occur by either transcellular or paracellular routes. The transcellular route involves translocation firstly across the apical or brush border membrane (BBM). The BBM has a high lipid content and depending on the nutrient involved, movement may occur via diffusion through the lipid environment or through polar regions and/or aqueous pores in the membrane (Csäky, 1984). In addition to diffusion or passive movement, carrier transport or pinocytosis may be involved. Each of these processes is described below.

The BLM has structural and morphological properties that are more similar to plasma membranes of nonintestinal cells. Again, movement through this membrane may involve diffusion, carriers, or pinocytosis (Csäky, 1984).

Intercellular or paracellular movement occurs via the junctional complexes (tight junctions) between adjacent intestinal cells (see section 1.10.6). The majority of intestinal electrolyte and water movement occurs through the lateral intracellular spaces between the cells (Armstrong, 1987). Solutes may also move through the paracellular route.

1.3.2) PASSIVE TRANSPORT/DIFFUSION

Simple diffusion involves the unrestricted movement of a solute caused solely by the kinetic energy or thermal agitation of the molecule. The rate of diffusional movement is dependent upon the concentration difference between two "compartments". In biological systems, the membrane between two compartments restricts free diffusion; thus, the rate of transfer is dependent upon the properties (resistance) of the membrane in addition to concentration differences. The resistance factor or permeability coefficient (expressed in cm/s) describes the amount of solute that crosses unit area of the planar cell membrane per

unit time per unit concentration of the solute and is characteristic of a select solute in a particular membrane (Thomson and Dietschy, 1984).

Passive net solute movement occurs in the direction of the electrochemical gradient across the intestine. In addition, passive transport is characterized by absence of competition between structurally related substances and lack of inhibition by metabolic inhibitors, anoxia, or absence of electrolytes. Often, passive movement is characterized by a linear relationship between transmembrane concentration difference and rate of transport. However, a linear relationship does not rule out other modes of transport (Thomson and Dietschy, 1984).

Lipids and lipid-substances are likely passively absorbed for the most part through the lipid environment of the membrane. However, additional carrier-mediated mechanisms may also be involved as described further in section 1.4.5.

Passive absorption of some solutes (e.g. amino acids and monosaccharides) may also occur through polar channels within the cell membrane or between adjacent cells. However, even in the absence of structured aqueous channels or pores, there is likely to be some passive movement due to the random movement of the hydrophobic chains of polar lipids in the membrane resulting in the formation of dynamic pores in the bilayer (Esposito, 1984). Thus, even for carrier-mediated nutrients (described below) there may be an important passive transport component, such as in the case of glucose (Debnam and Levin, 1975; Pappenheimer, 1990).

1.3.3) CARRIER-MEDIATED TRANSPORT

The precise molecular nature of carrier-mediated transport in the intestine is not conclusively defined. In general terms, carriers (presumably membrane bound glycoproteins) allow a polar substance to interrupt its hydrogen bonding with water to enter the lipid bilayer for transport across the membrane (Csäzky, 1984). Carrier-mediated transport is often

characterized by saturation kinetics (due to the limited number of carriers) and mutual inhibition of uptake by substrates of a similar structure (due to possible sharing of the same carrier).

Carrier-mediated transport may involve facilitated diffusion, in which case the carrier simply facilitates the movement of a substrate across the membrane. The facilitated transport exhibits diffusion kinetics (at concentrations below the saturation of the carrier) and thus net transport is discontinued when solute concentration becomes equal on either side of the membrane.

Carriers may also mediate active transport in which case a solute is transported against its chemical or electrochemical gradient. This process requires the expenditure of energy which is usually derived from the hydrolysis of compounds such as adenosine triphosphate (ATP). Other characteristics of active transport are described further in the context of hexose transport (section 1.6.5).

1.3.4) PINOCYTOSIS

Pinocytosis is a process involving the "infolding" of the cell membrane which detaches as an intracellular vesicle. The contents of the vesicle are then released into the cell cytoplasm accomplishing the transfer from the extracellular environment. The potential involvement of this process in the intestinal absorption of nutrients has not yet been fully defined (Csäky, 1984).

1.3.5) BARRIERS TO NUTRIENT UPTAKE

A) The Unstirred Water Layer

The various transport processes have been described. Clearly, these processes, in addition to the permeability of a solute and the membrane properties of the enterocyte are important determinants of the rate of uptake of nutrients. However, before crossing the

cells, solutes must first permeate a layer of unstirred water interposed between the membrane surface and the bulk luminal solution. This unstirred water layer (UWL) has been defined as "the concentric layers of water extending out from the aqueous-lipid interface that are not in equilibrium with the remainder of the bulk water phase" (Dietschy et al, 1971).

The effective resistance of the UWL is determined by the diffusion coefficient of the solute, the thickness of the unstirred layer and UWL surface area. The term "thickness" implies the layer of water adjacent to the cell membrane where diffusion is the only mechanism of transport. However, it is recognized that there is no sharp boundary where the bulk phase ends and the UWL begins; rather there is a progressive blending of the UWL with the bulk phase until they become indistinguishable (Dietschy et al, 1971).

Thomson and Dietschy (1984) have summarized the estimates of UWL dimensions from several studies. In highly stirred in vitro systems in rats and rabbits, values range from approximately 100-200 μm; unstirred preparations exhibit higher values of 200-350 μm. In vivo, UWL thickness is even higher ranging from approximately 400 to over 600 μm in rats and humans (Thomson and Dietschy, 1984). Thus, in the experimental situation the rate of stirring of the bulk phase affects the thickness and accordingly, the functional resistance of the UWL. In addition, the thickness of the UWL may also be affected in some physiological and pathophysiological states including thiamine deficiency, celiac disease (tropical sprue), drug-induced diabetes, aging and dietary manipulation (Thomson, 1982). It is possible that the alterations may be an adaptive mechanism to alter the absorption of nutrients (see section 1.7). The molecular mechanisms responsible for alterations in the UWL are not clear but do not appear to be related to changes in morphology of the intestine (e.g. height of the villi) (Thomson, 1982).

In an anatomically flat membrane, the surface of the UWL is equal to that of the underlying membrane. However, due to the complex morphology if the intestine, the UWL lies over and between intestinal villi and microvilli. Thus, assumptions of uniform thickness and surface area equivalent to that of the underlying membrane do not apply to the intestine. The effective surface area of the UWL relative to the underlying brush border is at least 1:500 (Thomson and Dietschy, 1984). Since molecules must first pass through the relatively small area of the UWL before reaching the greater area of the membrane, this has important implications on the overall rate of intestinal transport.

Thus, the movement of lipids and solutes must occur through two barriers in series; the UWL and the lipid membrane. When the diffusion coefficient is small relative to the permeability coefficient in the membrane, the UWL becomes the rate-limiting step for uptake during passive absorption (Thomson and Dietschy, 1981). Thus, for hydrophobic molecules including cholesterol and long chain fatty acids, this situation applies. Therefore, the UWL exerts a significant impact on the uptake of lipids and must be taken into consideration when determining rates of uptake in experimental situations.

The UWL may also significantly affect the apparent kinetics of carrier-mediated transport. The active transport of a substrate depends on the concentration immediately adjacent to the membrane. Since the UWL affects the rate of nutrient movement, the concentration of a molecule in the UWL is not necessarily equivalent to the concentration in the bulk phase. Therefore, in the experimental situation, failure to account for the presence of the UWL may result in an overestimation of apparent Km values and an underestimation of the passive permeability coefficient for a given probe (Dietschy et al, 1971).

B) Other Barriers to Uptake

In addition to the UWL, it is possible that the glycocalyx and intestinal mucus at the membrane interface also contribute to the overall resistance of nutrient uptake (Westergaard et al, 1986). It has been suggested that these parameters may alter the viscosity of the UWL, although the exact effect of mucus on the effective resistance of the UWL is not clear. In the experimental situation, mucus may result in trapping of a portion of the probe molecule in the adherent mucosal fluid. Therefore, a nonpermeable extracellular marker should be used to correct for the volume of mucus and other fluid which comprises the adherent mucosal fluid volume.

Mayer et al (1985) have suggested that a mucous material secreted by the intestine may bind cholesterol stoichiometrically prior to its transfer into enterocytes. This may represent a possible regulated barrier to cholesterol uptake in the intestine. However, there is no definitive general conclusion regarding the role of mucus and glycocalyx as barriers to lipid uptake and they may not significantly alter nutrient transport processes (Thomson and Dietschy, 1984).

1.4) DIGESTION AND ABSORPTION OF LIPIDS

1.4.1) DIETARY LIPIDS

Approximately 90% of the fat in our food is in the form of triglyceride (TG) (Kreutler, 1980). TG are triesters of glycerol with varying compositions of long chain fatty acids depending upon the dietary source. Animal products generally contain significant amounts of saturated (e.g. palmitic and stearic acid) and monounsaturated (e.g. oleic acid) fatty acids with smaller amounts of polyunsaturated structures. Vegetable products, with some exceptions, usually provide long chain-unsaturated fatty acids including oleic and

linoleic acid. The latter is an essential dietary component due to its lack of synthesis in the body. Although fatty acids rarely exist as individual species in dietary foodstuffs, they are the most abundant constituent of ingested lipids.

In addition to TG, the remaining 10% of dietary fat is provided primarily by cholesterol and phospholipids (PL). North American data of cholesterol intakes has indicated that consumption of this sterol ranges from 405 mg per day in males to 266 mg for females (Carroll et al, 1982). Based on average body weight, this corresponds to 5.79 mg/kg and 4.84 mg/kg in males and females, respectively. The majority of ingested cholesterol is in the unesterified form while approximately 10-15% is esterified with a long chain fatty acid. In addition to dietary intake, endogenous sources are provided by the cholesterol found in biliary secretions and sloughed endothelial cells which may account for 50% of the luminal load (Holt, 1972).

Estimates of dietary PL intake have ranged from 1-2 grams (Börgstrom, 1976) to 4-8 grams per day (Rizek et al, 1974). However, it is generally agreed that endogenous PL sources derived from biliary secretions and sloughing of epithelium provide the more significant contribution of 11-12 grams per day (Börgstrom, 1976; Noma, 1964). In fact, estimates of daily endogenous contributions have ranged as high as 22 grams (Carey et al, 1983). Over 90% of the biliary PL is phosphatidylcholine.

Chemically, lipids are a very heterogenous group and the ability of each lipid to associate with water and other lipids varies significantly. In addition to chemical classifications, lipids have been categorized according to the nature of their interactions with water (Small, 1968). Of the major dietary lipids, TG and cholesterol have been classified as insoluble, nonswelling amphiphiles, implying no interaction between these lipids and water in the bulk phase. PL, although still considered insoluble, have a greater ability to interact

with water in that they form a continuous bilayer or stable vesicle enclosing a water core (termed swelling).

The importance of the nature of these interactions becomes apparent in the following discussion of digestion, absorption and transport of these three major dietary lipids including TG, cholesterol and PL.

1.4.2) MECHANICAL AND CHEMICAL DIGESTION OF LIPIDS

The biological availability of lipids for absorption is influenced by the nature of their interaction with aqueous media and other lipids. Thus, an important goal of digestion is to transform the insoluble complex dietary and endogenous lipids into their more soluble components and therefore improve their bioavailability. This is accomplished by both mechanical and chemical means.

Mechanical effects play an important role in emulsifying and increasing the surface area of ingested fat. The chewing action in the mouth initiates this process and the subsequent peristalsis in the stomach and small intestine continues to further disperse the lipid droplets. In the acidic environment of the stomach the physical emulsifying effects may be enhanced by the presence of complex polysaccharides, products of peptic protein digestion and endogenous PL (Carey et al, 1983).

Chemical digestion of dietary lipids in adults is accomplished by four enzymes secreted into the intestinal tract. The progression of lipid through the gastrointestinal tract and the action of each of these enzymes will be discussed briefly.

Lipolysis of TG is initiated in the stomach: intragastric activity may account for up to one third of the total digestion of this lipid (Hamosh, 1973). The enzyme responsible for this lipolytic activity in humans originates mainly from the von Ebner's glands on the dorsal aspect of the tongue and is identified as lingual lipase or gastric lipase. The enzyme acts on

the fatty acid ester linkage of TG and appears to cleave the fatty acid in the sn-3 position preferentially, at a rate twice as fast as the sn-1 position. In addition, the enzyme is more active on medium and short chain TG fatty acids (Patton, 1981).

The major end products of the TG digestion by these lipases include diglycerides and fatty acids. Both of these end products are highly hydrophobic in the low pH environment of the stomach; thus, they likely partition into the core of TG (covered with a surface coat of mainly PL). A small fraction of TG (2-3%) and protonated fatty acids may also be present in the surface coat (i.e. at the lipid-water interface) (Carey et al, 1983).

These lipid particles in the chyme then enter the small intestinal lumen in small propulsed aliquots through the pyloric canal and are further emulsified by the shear forces of the propulsive movement. In the small intestine where most of the lipid digestion is completed, the emulsion is mixed with pancreatic juice containing additional lipases as well as bicarbonate and bile.

Pancreatic lipase is secreted by the acinar cell of the pancreas and functions at the TG-water interface. This enzyme hydrolyzes the primary (sn-1 and sn-3) ester bonds of TG to yield fatty acids and 2-monoglycerides (Tso, 1985). The presence of bile salts in the lumen (in concentrations close to and above "critical micellar concentration" see section 1.4.3) appears to physically prevent the binding of the pancreatic lipase to the TG substrate possibly by occupying the interface. In vivo, this inhibition is prevented by the presence of a protein cofactor designated "colipase" which is also present in the pancreatic juice (as a zymogen, procolipase which requires activation by trypsin). It is speculated that the colipase binds to the ester regions of the TG molecule in addition to forming a 1:1 lipase:colipase bond thus altering the conformation of lipase to promote substrate-enzyme interaction

(Börgstrom et al, 1979). This binding appears to be enhanced in the presence of lipid digestion products.

Carboxyl ester lipase (also termed cholesterol esterase, nonspecific lipase and others) is also secreted in the pancreatic juice. It has been shown to catalyze the hydrolysis of water-soluble carboxyl esters as well as insoluble esters including cholesterol and vitamins A, D and E. The enzyme is secreted in an inactive monomeric form that requires bile salts to dimerize to the active form (Carey et al, 1983; Börgstrom, 1988).

Phospholipase A_2 is also present in the pancreatic secretions in the proenzyme form. Upon activation by tryptic hydrolysis, this enzyme catalyzes the hydrolysis of the fatty acids at the sn-2 position in phosphatidylcholine and a variety of phosphoglycerides (excluding sphingolipids), resulting in the formation of lysophospholipids and fatty acids. Bile salts and Ca ions are required to promote this hydrolytic activity. Although other phospholipases may hydrolyze PL at alternate positions, the actions of phospholipase A_2 appears to be the most physiologically important (Shiau, 1987).

The coordinated presence of the lipids, pancreatic secretions, bicarbonate (to raise the pH to 5.5-6.5 to permit effective action of lipolytic enzymes) and bile salts is important to accomplish the task of digestion. Therefore, although each of the lipolytic enzymes has been discussed separately above, it is clear that cooperation and synergism play an important role. For example, the partial hydrolysis of TG in the stomach with the resulting 15-20% free fatty acid content (Börgstrom, 1988) has been suggested to enhance the subsequent hydrolytic action of pancreatic lipase in the upper small intestine due to the stabilization of the surface of the TG emulsion and increased binding of colipase (and thus lipase) at the interface (Carey et al, 1983). Furthermore, the presence of lipid in the intestinal lumen, particularly long chain fatty acids, appears to stimulate the release of the hormone cholescystokinin-

pancreozymin which in turn induces contraction of the gallbladder and simultaneous relaxation of the sphincter of Oddi, with the resulting secretion of bile and the discharge of pancreatic lipases.

1.4.3) SOLUBILIZATION OF HYDROLYZED LIPID PRODUCTS

The combined effects of the lipolytic activity on the non-polar dietary lipids results in the production of mainly fatty acids and 2-monoacylglycerols, in addition to lysolecithin and free cholesterol. Although the end-products in general are more polar than the original ingested forms, their aqueous solubility is still limited. Therefore, the second important phase of digestion is the dispersion of these hydrolyzed products into absorbable forms (Patton 1981).

The interaction of lipolytic products with bile acids in mixed aggregates or micelles is very important to improve their interaction with water and thus their rate of absorption. Bile acids are end products of cholesterol metabolism synthesized by the liver. The conjugation of the hydrophobic bile acid steroid nucleus with amino acids (taurine or glycine) and the presence of hydroxyl groups (in varying numbers and positions) account for the amphipathic nature of these soluble compounds and their ability to stabilize at an oil-water interface. At low concentrations bile salts exist as monomers in solution. However with increased concentrations above a given point (referred to as critical micellar concentration) spontaneous aggregation occurs resulting in the formation of micelles with the polar groups exposed to the aqueous phase, making the entire aggregate water soluble. These negatively charged micelles have the ability to solubilize lipids, particularly swelling compounds including PL and monoglycerides, between the amphipathic bile acids or into the hydrophobic centre. The addition of swelling amphiphiles to bile salt micelles enhances the solubilization of

nonpolar lipid compounds into the aggregate. For example, in the presence of PL and monoglyceride, cholesterol solubilization is enhanced (Shiau, 1987).

As indicated earlier, the presence of lipids in the proximal intestine stimulates the secretion of bile (i.e. a micellar solution of bile acids, phosphatidylcholine, and cholesterol) which results in luminal bile acid concentrations well above critical micellar concentration. The micelles, containing bile salts and the biliary lipids, solubilize the products of lipolysis as described above.

Thus, the lipid digestive products become distributed between an aqueous, oil and possibly several intermediate phases in the intestinal lumen. The aqueous phase is believed to include the mixed micelles and monomeric forms of the digested lipid products, in equilibrium with the aggregated products. It is suggested that these micelles coexist with liquid crystalline vesicles saturated with bile salts in the aqueous phase (Carey et al, 1983), although little is known about the role of these dispersions on the uptake of lipids in the normal physiological setting (Borgstrom, 1988).

1.4.4) MOVEMENT OF LIPIDS INTO THE ENTEROCYTE

The solubility of a lipid in the bulk phase will influence its concentration gradient across the intestinal cell. These factors, in addition to the passive permeability coefficient of the lipid for crossing the membrane are important determinants influencing the overall rate of uptake. However, as described in section 1.3.5, due to the low diffusion coefficient of cholesterol and fatty acids, the UWL is likely the rate limiting step in the uptake of these hydrophobic structures.

The presence of bile acids, particularly trihydroxy forms in the lumen, is obligatory for the absorption of cholesterol, whereas approximately one third of TG and some fatty acids may be absorbed in the absence of micellar solubilization (Thomson and Dietschy,

1981). The role of the bile salt micelle in cholesterol uptake has been defined as a means of solubilizing cholesterol, as well as overcoming the resistance of the UWL. In addition, micelles are believed to provide a reservoir from which cholesterol partitions into an aqueous phase prior to its uptake into the membrane (Westergaard and Dietschy, 1976). Biliary cholesterol may be more efficiently absorbed than dietary cholesterol due to existing micellar solubilization in the bile (Grundy, 1983).

The concentration of lipolytic products in the aqueous environment is increased 100 to 1000 times by micellar solubilization, in spite of the increase in diffusional UWL resistance to the relatively large aggregated form of the micelle (Thomson and Dietschy, 1981). As indicated above, the passage of smaller molecular weight substances including short and medium chain TG's, is not rate-limited by the UWL.

Both the monomeric and aggregated forms of lipid move towards the BBM for absorption. However, it is generally concluded that mixed micelles are not absorbed intact but must dissociate before the lipids permeate the enterocyte. Experimental evidence indicating that the components of mixed micelles are absorbed at different rates supports this view. There is strong experimental evidence to suggest that fatty acids and cholesterol solubilized in the micelle are released into the aqueous phase as monomers which partition (according to individual permeability characteristics) into the adjacent membrane of the absorptive cell (Thomson and Dietschy, 1981). The postulated acidic micro-environment adjacent to the cell membrane likely facilitates the dissociation of micelles. The low pH presumably favours the protonation of fatty acids in the micelles resulting in their reduced solubility in the aggregate and enhanced partitioning into the membrane (Shiau, 1987). The resulting micellar disaggregation likely enhances the release of other lipids (e.g. cholesterol) from the micelle.

When increasing concentrations of the bile acid taurodeoxycholic acid (TDC) are used to solubilize cholesterol in the bulk phase, there is a progressive decline in cholesterol uptake (Thomson, 1981; Thomson et al, 1983). When the concentrations of both TDC and cholesterol were increased but the ratio of TDC to cholesterol remained constant, the rate of cholesterol uptake remained unchanged. The bile salts may favour retention of the cholesterol in the micellar phase and decrease the monomeric concentration available for uptake. However, this latter relationship is not observed in mixed micelles, and therefore may not be significant physiologically. Thus, the components of the micelle, as well as the shape, size and the charge of the aggregate will all influence the movement of the lipid from the micelle to the membrane.

Traditionally, it has been suggested that the lipophilic nature of the fatty acids allowed their direct passive diffusion through the phospholipid bilayer of the mucosal cell. However, for some long chain fatty acids (oleic, linoleic and arachidonic acid) saturatable, concentration-dependent absorptive trends have been observed (Hollander et al, 1984; Stremmel, 1985; Stremmel, 1988). Furthermore, high affinity binding sites for long chain fatty acids in the BBM of the jejunum have been reported. In addition, a 40-kD membrane fatty acid binding protein (MFABP) in these membranes has been described and characterized by Stremmel and co-workers (Stremmel, 1985, 1988). Thus, the saturable kinetics and the inhibition of fatty acid uptake by a monospecific antibody against MFABP support the suggestion that fatty acid uptake is at least in part, mediated by a membrane carrier (Stremmel, 1985, 1988). The antibody inhibition studies carried out in isolated perfused jejunal segments of rats also demonstrated inhibition of uptake for palmitate, linoleate, arachidonate and cholesterol. Stremmel suggests that the MFABP may therefore exhibit transport competence for other lipolytic products. As described below, fatty acid

binding proteins (FABPs) are also shown to be present in the cytosol of several cells including enterocytes.

1.4.5) INTRACELLULAR EVENTS

Once the lipid products of digestion have permeated the cell they are transported through the cytoplasmic matrix to the endoplasmic reticulum (ER), which is the major site for metabolism of absorbed lipids. Cytosolic proteins (FABPs) with a high affinity for fatty acids (particularly long chain and unsaturated) may play a role in transporting the fatty acids from the BBM to the ER. However, the precise role for FABPs in the uptake, intracellular targeting, and metabolism of fatty acids has not been defined (Lowe et al, 1987).

Intestinal tissue has two distinct FABPs including liver-(L-FABP) and intestinal-type (I-FABP). The highest concentration of I-FABP appears to be located in the villi of the jejunum and is not expressed in crypt cells, thus implying that FABP expression is an event of differentiation (Clark and Armstrong, 1989). It is unclear why two cytosolic FABPs are present in the intestine. Lowe et al (1987) suggest that the two types differ in their affinities for various fatty acids as well as in transport capacity. They hypothesize that I-FABP may direct and transport saturated fatty acids absorbed into the enterocyte while L-FABP, with a higher affinity for unsaturated fatty acids, might target endogenous (bloodstream) fatty acids to phospholipid synthesis. FABPs may play a role in enhancing free fatty acid uptake by facilitating desorption from MFABP (Clark and Armstrong, 1989). It is also speculated that these proteins may influence various cellular lipid metabolism functions. However, conclusive evidence regarding the varying affinities of FABPs for different fatty acids as well as the actual function of FABPs remains speculative at this point and further study is required.

The transport mechanisms responsible for the other digested lipids within the cell remains undefined.

At the ER, TG may be resynthesized from fatty acids and monoglycerides via the monoglyceride pathway or from fatty acids via the α-glycerophosphate or phosphatidic acid pathway. The initial step in both pathways involves the activation of fatty acid to acyl-CoA - a reaction requiring ATP which is derived mainly from glucose metabolism. The monoglyceride pathway is more important during lipid digestion and may account for more than 70% of total intestinal TG synthesis under physiological conditions (Shiau, 1987). During fasting, the phosphatidic pathway predominates, using endogenous fatty acids and glucose metabolites as substrates. It has been suggested that these two pathways function independently and that the intermediate (i.e. diglycerides) and end-products (TGs) of each remain in separate pools in the intestinal mucosal cell; the exact mechanisms and functional significance of this separation remain unclear (Tso, 1985).

Absorbed fatty acids may also be used for reacylation of PL and esterification of cholesterol as will be discussed. Recent evidence suggests that desaturase enzymes are present in the enterocyte; thus, some fatty acids may provide substrates for the de novo synthesis of other polyunsaturated fatty acids that eventually become components of the lipid membrane (Garg et al, 1988). Fatty acids may also be metabolized via the oxidative pathway when availability of glucose is reduced.

Absorbed lysophospholipids, mainly lysolecithin, are acylated by acyl-CoA to form PL. Lysolecithin may also be hydrolyzed by lysolecithinase to release glycerylphosphorlycholine and fatty acid.

Cholesterol absorbed from the lumen appears to combine with the endogenous pool of cholesterol consisting of products of de novo synthesis and cholesterol derived from

lymphatic lipoproteins. The absorbed free form of cholesterol may be esterified by acyl-coenzyme:cholesterol acyltransferase (ACAT) or by the pancreatic cholesterol esterase within the enterocyte. It is estimated that 80-85% of the cholesterol appearing in the lymph (in chylomicrons [CM] and very low-density lipoproteins) is esterified; thus esterification appears to be important for cholesterol transport although the rate limiting step is likely the uptake from the lumen (Thomson, 1982). The fatty acids used for esterification are influenced by the composition of the dietary lipids (Thomson et al, 1989a), however, it has been suggested that oleic acid is the preferred fatty acid for esterification (Karmen et al, 1963).

CCBs have been shown to inhibit cholesterol esterification in cultured macrophages possibly by a direct action of these agents on the ACAT enzymes (Daugherty et al, 1987). It is unknown whether a calcium dependent step is involved in the regulation of ACAT, but it is likely that inhibition of this enzyme would influence intestinal lipid uptake (Tso, 1985).

The resynthesized lipid products in the cell are mainly nonpolar and nonswelling in nature (i.e. cholesterol esters [CE] and TG); thus, once more their solubility must be enhanced for aqueous transport. The solubilization of lipids for transport in the lymphatics and plasma is accomplished by incorporation into macromolecular complexes called lipoproteins. The general model is a spherical complex containing mainly TG and CE in a hydrophobic core surrounded by a layer of PL, free cholesterol and small amounts of specific proteins (apoproteins) and likely a trace of the hydrophobic core lipids. CM are the principle lipid-carrying particles secreted by the small intestine following lipid digestion. The composition and metabolism of CM and other intestinal and hepatic lipoproteins will be discussed in the following section.

The sites and mechanisms for assembly of lipoproteins in the enterocyte are not completely delineated. Ultrastructural studies have shown that following the ingestion of fat, droplets of lipid appear within cisternae and vesicles of smooth ER in the apical cytoplasm of enterocytes. The vesicles then appear to move sequentially through the cell, likely through a continuous tubular system to the Golgi apparatus. The Golgi cisternae become distended with the droplets and pinch off to form vesicles which then migrate toward and fuse with the plasma membrane. The lipoprotein complexes are then released into the lacteals of the lamina propria and eventually enter the venous system via the thoracic duct.

The lipid-protein complex is continually modified during the progression through the cell as protein and carbohydrate moieties are added at various stages. Furthermore, the completed lipoprotein is modified almost immediately after secretion into the extracellular environment. The significant processes are discussed in the following section.

1.5) LIPOPROTEIN METABOLISM

1.5.1) GENERAL

As indicated above, lipoproteins are macromolecular complexes that transport lipids in the aqueous environment of the body. Although lipoproteins are in a constant state of exchange and modification they are most commonly classified according to their hydrated densities. The five classes of lipoproteins include CM, very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), low density lipoproteins (LDL) and high density lipoproteins (HDL). Only the liver and the small intestine are capable of directly secreting the lipoproteins. However, a number of important metabolic alterations take place in the plasma.

1.5.2) APOPROTEINS

The apoproteins associated with the lipoproteins are synthesized by the liver and the small intestine, although most are derived from circulating lipoproteins or their remnants in plasma (Shiau, 1987). Specific apoproteins are associated with the lipoproteins via hydrophobic interactions. However, due to the amphipathic nature of the amino acid sequence, hydrophillic portions of the protein are directed toward the surface of the particle. Thus, the apoproteins behave like peripheral proteins participating in transfer and exchange between lipoprotein classes. The protein content of the lipid-carrying particles represents a very small percentage of the total mass (e.g. 1-2% by weight for CM [Tso, 1985]), however their presence in the lipoprotein is a critical factor in their transport and metabolism. Using the ABC nomenclature proposed by Alaupovic (1972) several apoproteins have been classified and will be referred to in the context of their roles as cofactors, and inhibitors of lipoprotein metabolism.

1.5.3) TRIGLYCERIDE RICH LIPOPROTEINS

The major TG carrying lipoproteins are the CM secreted from the small intestine after lipid ingestion and VLDL secreted from the liver. The rate of secretion is dependent upon TG synthesis in both of these tissues. The CM are the most important carrier of TG derived from luminal absorption although endogenous sources may also be carried (Shiau et al, 1985). They are the largest lipoprotein with an average diameter of 1200 Å, although this varies significantly according to the amount of lipid carried in the core. The composition of CM particles is estimated to be 86-92% (of total mass) TG, 0.8-1.4% CE, 0.8-1.6% free cholesterol, 6-8% PL and 1-1.5% protein (Shiau, 1987).

The VLDL secreted from the liver transport TG synthesized mainly from endogenous fatty acid sources, including albumin-bound free fatty acids from the adipose tissue during

the interprandial period. Following ingestion of fat, a variable portion of the CM TG (hydrolyzed by the action of lipases) may also contribute to the fatty acid pool in the liver. Furthermore, excess dietary carbohydrate may be converted to free fatty acids in this tissue (Havel et al, 1980).

The intestine also secretes VLDL with a similar lipid and protein composition to CM but a smaller size (280-750 Å) (Ockner et al, 1969). In the fasting state, VLDLs are the major lipoprotein in the intestinal lymph. Based on a variety of experimental observations Tso and Simmons (1984) have proposed that TG formed via the aforementioned monoglyceride pathway are carried predominantly in CM whereas TG synthesized by the α-glycerophosphate pathway are packaged into VLDL. Although not yet resolved, most recent studies appear to support the notion that intestinal VLDL are formed independently of CM via separate pathways and mechanisms, rather than simply being smaller CM.

Both CM's and VLDL contain apoprotein B (apo-B) as an essential structural component. The larger apo-B of hepatic origin associated with VLDL has been arbitrarily called apoB-100 and the smaller form produced in the intestine (estimated to be 48% of the unresolved molecular weight of the hepatic form) has been designated apoB-48. Unlike the other apoproteins, apo-B does not exchange between lipoproteins and remains an important component of the lipoproteins during their entire metabolism. Based on ultrastructural studies it appears that when apo-B is prevented from being incorporated, transport of the lipid particles within the enterocyte is inhibited (Gotto et al, 1971).

The initial stages of the metabolism of CM's and VLDL are similar. Immediately after secretion there is a rapid exchange of apoproteins between the newly formed (nascent) particles and the circulating HDL. The HDL transfer apoprotein-E (apo-E) to the CM's and both VLDL and CM acquire C apoproteins. The nascent CM are enriched in apoproteins

A-I and A-II, however these are rapidly transferred to HDL particles in the plasma (Anderson et al, 1981).

Apo-CII (received from HDL) plays a major role in the initial TG-rich lipoprotein degradation. This protein has been shown to accelerate the activity of lipoprotein lipase, an important enzyme in lipid metabolism, responsible for the hydrolysis of TG in both CM and VLDL. The active forms of the enzyme are located extracellularly at the surface of capillary endothelia and appear to be most effectual in capillaries of adipose tissue, cardiac and red skeletal muscle and lactating mammary gland (Havel et al, 1980). The precise nature of the interaction between lipoprotein lipase and the TG substrate has not been determined, however the activity of the enzyme ultimately results in the removal of TG from the core of the particle, producing remnant particles. The end products of the lipase activity, mainly fatty acids, are either taken up by associated tissue or bound to albumin for transport to other tissues, depending upon the physiological state. The continual removal of the lipoprotein components, and in particular apo-CII results in a progressive reduction in the hydrolytic activity and when 80-90% of the core components have been broken down, lipoprotein lipase activity is minimal (Higgins and Fielding, 1975).

As the TG is removed from the cores of CM and VLDL they acquire CE from circulating HDL (as described in section 1.5.5) and the ratio of apo-E:apo-C in the particle rises as apo-C is lost to HDL. The net result of these changes is a smaller particle with reduced TG:cholesterol ratio designated "chylomicron remnants" from CM and "IDL" or "VLDL remnants" from VLDL. These particles maintain the original content of apo-B, in addition to some apo-C and an enriched content of apo-E, all of which are important for the subsequent catabolism of these lipoproteins by the liver.

CM remnants are actively cleared from the plasma by uptake via a specific hepatic receptor, that recognizes the presence of apo-E and appears to be inhibited by the presence of apo-CIII (Calvert and Abbey, 1985). The receptor mediates an endocytotic process resulting in delivery of CM components to the hepatocyte.

The major catabolic route for the VLDL remnants is hepatic endocytosis via the well characterized LDL receptor mechanism (apo B-100,E receptor described further in the following section). In humans it has been suggested that the apo-E receptor for CM may also bind large VLDL remnants. The VLDL remnants that are not internalized by the liver remain in the plasma where most of the remaining TG is hydrolyzed possibly through the action of hepatic TG lipase in the liver sinusoids. The particles are also further depleted of CE by an unknown mechanism. The resulting particles with a core composed of mainly CE and containing apo B-100 as the only apoprotein are designated as LDL.

1.5.4) LOW DENSITY LIPOPROTEINS

Studies in normal humans and rats have concluded that all of the LDL in plasma is derived from the metabolism of VLDL. However the fraction of VLDL converted to LDL varies significantly among species, and this difference and the determinants of VLDL to LDL alterations are not well understood. In humans it is estimated that approximately half of the VLDL is taken up while the remaining portion is converted to LDL. In rabbits approximately 10% of VLDL forms LDL and in rat the value may be 5% or less (Havel, 1987).

In addition to this variation between different species, the lipoprotein composition may also differ. For example, in humans, LDL is the primary carrier of excess plasma cholesterol. However, rabbits, which exhibit an extreme sensitivity to dietary cholesterol, carry cholesterol in β-VLDL particles (Kovanen et al, 1981). These β-VLDL particles differ

from normal VLDL particles which carry TG as the major lipid component (section 1.5.3). In addition, the apoprotein content and electrophoretic mobility differ between the two lipoproteins. The rapid hypercholesterolemic response observed in rabbits may be due to enhanced absorption of dietary cholesterol and a failure to develop high rates of bile acid production in response to cholesterol feeding.

The removal of LDL from the circulation occurs mainly by the LDL receptor mechanism. Since the LDL contain only apo-B-100 they compete poorly with the apo-E containing VLDL remnants for the receptor and therefore circulate in the plasma for longer periods (Havel, 1987). The receptors were first described in cultured human skin fibroblasts in 1974 by Brown and Goldstein (summarized in Goldstein and Brown, 1975) and have subsequently been identified in all other tissues studied with the exception of the nervous system. The receptor, a 5-domain protein, localizes at specialized regions of the plasma membrane (coated pits) that facilitate invagination and internalization of the receptor-lipoprotein complex to form endocytotic vesicles. The formed vesicle then migrates through the cytoplasm of the cell where it fuses with a primary lisosome to form a secondary lisosome in which the apo-B is degraded and the CE is hydrolyzed.

The free cholesterol derived from LDL mediates several important actions to stabilize the cholesterol content of cells including: suppression of cholesterol biosynthesis by inhibiting the rate-controlling enzyme 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), activating ACAT to re-esterify excess cholesterol for storage, and suppressing the synthesis of LDL receptors, thus controlling cholesterol entry into the cell (Goldstein and Brown, 1975).

As indicated earlier, it is undetermined but possible that calcium may influence the activity of ACAT. Furthermore, a calcium and calmodulin dependent kinase has been

suggested to play a role in the short-term regulation of HMG-CoA reductase (Beg et al, 1987a, 1987b). Reduced calcium influx by CCBs may upregulate HMG-CoA reductase activity by promoting dephosphorylation of HMG-CoA reductase. The resulting enhanced cholesterol synthesis would likely influence cholesterol uptake as well as membrane lipid composition (Brasitus and Schacter, 1982).

Furthermore, it has been observed that in vitro CCBs stimulate the synthesis of LDL receptors, another factor which may have important implications for cholesterol uptake (Paoletti et al, 1988).

In addition to LDL receptors it has been demonstrated that part of LDL catabolism is mediated by lower affinity routes that may or may not be receptor independent. Based on data comparing [^{125}I]-LDL turnover rates in individuals with receptor deficient cells (i.e. homozygotes for familial hypercholesterolemia) to turnover rates in normolipemic humans it is estimated that these "alternate" pathways may account for one-third of the LDL catabolism in the normal state (Havel, 1980). Although there are limitations when making such comparisons the majority of studies support the observation that the majority of LDL uptake is receptor mediated. Dietschy (1984) suggests that the importance of the LDL receptor uptake varies for specific organ systems. For example, in the liver, endocrine glands, lung and kidney, receptor-dependent LDL transport may account for more than 90% of the LDL uptake. However, in the small intestine and spleen, receptor-independent mechanisms may account for nearly three-fourths of the observed uptake.

The relative importance of the receptor-dependent and receptor-independent mechanisms in each organ may vary depending on changes in the plasma LDL levels. Kinetic-transport data for LDL transport have been calculated for several animal species as well as humans (Meddings et al, 1987). Thus, while LDL receptor mediated uptake has

gained much attention, receptor independent LDL uptake may be quantitatively significant. It is unknown whether calcium or CCBs modify receptor-independent transport. The significance of LDL uptake by alternate routes will be discussed more fully in the context of atherosclerosis (see section 1.11.2).

1.5.5) HIGH DENSITY LIPOPROTEINS

There are several discrete classes of HDL. These have been categorized into HDL_1, HDL_2 and HDL_3 subgroups with the latter two being the major forms in human plasma. Interconversion occurs between the latter two groups which differ mainly in their content of CE. HDL_1 is a major particle in rat plasma but has also been reported in humans (Mahley, 1982). In animals fed a cholesterol rich diet HDL_c has been identified and is likely an analogue of normally circulating HDL_1 developed to carry the large amounts of cholesterol.

HDL may be secreted directly by the small intestine and the liver, although some authors have challenged the suggestion that these are true secretory products (Eisenberg, 1984). The excess surface material released during the degradation of CM and VLDL (i.e. free cholesterol, PL and apoprotein C) form precursors for HDL particles. The challenging authors suggest that the immediate initiation of lipolysis of newly formed CM (possibly in the lacteals) and VLDL release these surface constituents and that these represent the so called "secreted HDL". Thus, surface constituents would represent the major, if not the only, precursor of HDL.

The nascent HDL particles are very different from those circulating in plasma. In general terms, the secreted HDL are in a discoidal form and consist mainly of a bilayer of PL and unesterified cholesterol with no CE and apo-E as the major apoprotein. The HDL in plasma are spherical, have a higher content of CE and apo A-I. The nature of HDL particles secreted by the intestine is unclear. Both discoidal and spherical particles appear

in the mesenteric lymph of rats but discoidal forms have not been identified in normolipemic humans.

The nascent forms of discoidal HDL appear to be transformed to the spherical form in plasma by the activity of lecithin-cholesterol acyl transferase (LCAT) (Shiau, 1987). LCAT is produced in the liver and its activity as an acyltransferase, resulting in the formation of CE from phosphatidylcholine, is dependent upon the presence of apo-AI, the major apoprotein component of most particles in the HDL density class (Eisenberg, 1984). Evidence for the importance of apo-AI and LCAT is provided by the observation that subjects with Tangier disease (Apo-AI deficient) and familial LCAT deficiency do not transform nascent HDL (Calvert and Abbey, 1985).

As HDL cholesterol is the preferred substrate for the activity of LCAT, a major role of HDL is to function as a site for cholesterol esterification in the plasma. The PL and CE components of HDL are in continual exchange between lipoproteins and cell membranes. In humans, most LCAT derived CE is transferred to other lipoproteins of lower density (Eisenberg, 1984). The transfer of CE is facilitated by a protein present in the plasma referred to as plasma lipid transfer protein. Although this protein participates in the movement of TGs and PL it's major role in humans is believed to be the transfer of esterified cholesterol from the HDL.

In addition to cholesterol esterification, HDL essentially acts as a reservoir for lipid and apoproteins as well as interacting with the lipases, the LCAT system and the lipid transfer proteins. In fact, some authors have suggested that LCAT and transfer proteins are actually a component of a specialized subfraction of HDL.

As first proposed by Glomset in 1968, HDL also appears to play a major role in the movement of free cholesterol molecules from most cells to the liver and other tissues (i.e.

reverse cholesterol transport). This ability has been attributed to a very small fraction of HDL_3 with a specific content of apo-AI. There appear to be HDL binding sites on many cells that facilitate this process. LCAT likely enhances the ability of HDL to accept free cholesterol, especially when lipid transfer proteins and acceptors of the CE are present. The binding sites also recognize a variety of proteins and lipoproteins including LDL but the exact nature of the receptors still needs to be clarified.

The mechanisms responsible for the catabolism of HDL are not well understood. HDL appears to have several modes of interaction that vary between cells. As indicated above HDL binding is not necessarily followed by catalytic events as in the case of LDL. Recent evidence suggests that the apoprotein components and cholesteryl esters are metabolized separately (Eisenberg, 1984). The mechanisms and significance of these observations are yet to be defined.

1.5.6) DIETARY INFLUENCES ON LIPOPROTEIN METABOLISM

A) Variability in Responses

Dietary components may have a significant effect upon serum lipoprotein concentrations in humans and animals. However, as indicated earlier, not all species respond to dietary influences in a similar manner. As discussed, the rabbit is particularly sensitive to dietary cholesterol intake and transports the excess cholesterol in a unique β-VLDL lipoprotein particle.

The magnitude of response to dietary components may also be highly variable within a species. For example, responses in serum cholesterol may exhibit wide inter-animal differences in cholesterol fed animals and to a lesser degree in humans (Beynen et al, 1987). Individuals showing only small alterations in serum cholesterol have been designated "hyporesponders" and those developing significant hypercholesterolemia have been called

"hyperresponders". A similar phenomenon has been described in response to dietary saturated fatty acids in humans and rabbits (Grundy and Vega, 1988).

The mechanisms responsible for the variability in individual serum responses to dietary components has not been determined. Beynen et al (1987) propose that hyperresponders may produce greater LDL (or precursors for the same) than hyperresponders) possibly due to enhanced cholesterol absorption compared to hepatic hyporesponders. Alternatively, altered regulation of cholesterol biosynthesis could play a role. It is likely that there is a genetic component although much further study is needed to improve in identifying hypo- and hyperresponders as well as to determine the underlying mechanism(s).

B) Dietary Components

Two important dietary factors influencing plasma lipids and lipoproteins include cholesterol and fat; both the total fat content as well as the types of dietary fat are important. The amount of cholesterol and fat in the diet may affect hepatic lipid and lipoprotein synthesis as well as influence the activity of the LDL receptor.

For example, dietary cholesterol is taken up by the liver as a component of chylomicron remnants (see section 1.5.3). Thus, dietary cholesterol contributes to the total amount of cholesterol in the hepatic cell and thus may decrease hepatic receptor LDL activity as well as influence cholesterol biosynthesis. Meddings et al (1987) quantitatively demonstrated that cholesterol feeding induced a rise in plasma LDL levels in hamsters due to an increase in LDL production rate coupled with decreased maximal transport rates for receptor-mediated LDL uptake. Dietary saturated fats appeared to further suppress hepatic LDL receptor activity and removal of plasma LDL (Grundy and Denke, 1990; Meddings et al, 1987). High intakes of saturated fatty acids reduce mRNA for LDL receptors in baboon

liver (Fox et al, 1987). Saturated fats may also directly increase the synthesis of cholesterol and LDL in the liver (Connor and Connor, 1990). However, not all saturated fats are hypercholesterolemic. For example, stearic acid (18:0) does not significantly influence serum cholesterol levels, likely due to its conversion to the monounsaturated species, oleic acid (18:1), via a desaturase enzyme (Connor and Connor, 1990). Monounsaturated fatty acids have a reportedly neutral affect on serum cholesterol although recent research has postulated a cholesterol lowering effect (Grundy, 1986). Whether the reduction in serum cholesterol level occurs due to a direct induction of LDL receptors by the monounsaturated fatty acid or a secondary effect due to the removal of saturated fats from the diet is unclear (Grundy and Denke, 1990).

Polyunsaturated fatty acids include species from the omega-6 and the omega-3 branches. The essential fatty acid linoleic acid (18:2), and arachidonic acid (20:4) (synthesized in liver or from linoleic acid) are from the former class while linolenic acid (18:3), eicosapentaenoic acid (20:5), and docosahexaenoic acid (22:6) are omega-3 fatty acids. The latter two are derived from fish, shellfish (particularly in fish oils) while the former is found in some vegetable products. Either class of fatty acid has been shown to depress plasma total and LDL cholesterol levels. Unlike the omega-6 polyunsaturated fatty acids the omega-3 fatty acids may affect several other factors related to the pathogenesis of atherosclerosis by modifying vasoconstriction, platelet aggregation, blood viscosity, leukocyte function, and several other postulated effects (Connor and Connor, 1990).

1.6) CARBOHYDRATE DIGESTION AND ABSORPTION

1.6.1) DIETARY CARBOHYDRATE

Traditionally, the majority of carbohydrate consumed by human adults has consisted of polysaccharides (starch) with a lesser portion being provided by disaccharides (sucrose and lactose) or monosaccharides (glucose and fructose). However, with increased consumption of refined and processed foods in Western society, the latter two groups (simple sugars) may provide up to half of the estimated 300 grams of carbohydrate ingested daily (Alpers, 1987). Small, variable amounts of oligosaccharides (raffinose, stachyose) and polysaccharides (cellulose and others) that are resistant to digestion are also consumed.

1.6.2) STRUCTURE OF CARBOHYDRATES

Ingested starch consists of linear chains of glucose linked between carbon 1 of one glucose molecule and carbon 4 of a second unit (i.e. 1-4 linkage in α configuration). In addition to the straight-chain glucose polymers (termed amylose), branched linkages of glucose occur via linking between carbons 1 and 6. The branched structure is called amylopectin and is the second major component of ingested starch. These α-1-6 branching points occur approximately every 20-25 glucose residues along the amylose chain and thus join adjacent α-1-4 chains (Gray, 1981).

The major ingested disaccharides include sucrose, an α-linked glucose and fructose molecule, and lactose which consists of galactose and glucose in a β-1,4 linkage.

Before being assimilated by the body these dietary forms are first hydrolyzed to their monosaccharide derivatives which are then transported by specific mechanisms across the enterocyte. The nature of the linkage between monosaccharide units, as well as the structures involved have important implications for the digestive process. The digestion of

carbohydrate will be briefly reviewed before providing a more in-depth view of absorption, with an emphasis on glucose and galactose uptake mechanisms.

1.6.3) DIGESTION OF CARBOHYDRATES

The digestion of starch is initiated in the mouth by an endo α-1-4 glucosidase called α-amylase. This enzyme acts to randomly hydrolyze the interior α-1-4 bonds of the amylose chain. The α-1-6 linkages, as well as the α-1-4 linkages adjacent to the branching points are resistant to the hydroiytic activity of α-amylase. In the acidic environment of the stomach, this enzyme is denatured, although the presence of short chain substrates may provide protection against complete inactivation. Thus, in the stomach, hydrolysis is limited to the action of residual salivary amylase and some acid hydrolysis (Reiser and Lewis, 1986).

The majority of starch digestion occurs in the intestinal lumen by pancreatic α-amylase which may also be attached to the surface of the enterocyte by electrostatic charge (Alpers, 1987). This enzyme acts in a manner similar to the salivary amylase (i.e. cleaving α-1-4 bonds and is also ineffective against the α-1-6 branched points). Thus, the major end-products of the combined hydrolysis include chains consisting of 5-10 glucose residues including 1 or more α-1-6 branched links; the latter structures are called α-limit dextrans. In addition, α-1-4 linked double or triple units of glucose (maltose, and maltotriose, respectively) are formed. The hydrolysis is rapid with the three major end products appearing in approximate equal amounts by the duodenal-jejunal junction (Gray, 1981).

As indicated earlier, the nature of the linkages within polysaccharides is an important determinant of the hydrolytic activity by carbohydrases. For example as indicated, the amylases are ineffective against the α-1-6 linkage of amylopectin. Furthermore, glucose units linked by a β linkage between carbons 1 and 4 (β-1-4 linkage) are also resistant to hydrolysis.

As a result, cellulose and its analogues are considered indigestible by humans and are collectively referred to as dietary fibre.

The final digestive step of carbohydrate occurs by membrane-bound enzymes in the BBM. Hydrolytic sites of these "oligosaccharidases/ dissaccharidases" are exposed at the luminal surface to act upon the end-products of the amylase digestion as well as ingested disaccharides. These enzymes may act on specific bonds occurring between particular substrates. For example, the brush border enzyme lactase acts specifically to cleave lactose into its respective monosaccharides of glucose and galactose. Sucrase hydrolyzes the α-1-4 linkage of sucrose resulting in the release of free glucose and fructose. However, the sucrase enzyme also plays an important role in hydrolyzing the α-1-4 bonds of maltose and maltotriose. Isomaltase or α-dextrinase is essential for hydrolyzing the α-1-6 glucose linkages from partially hydrolyzed α-limit dextrins. Trehalase acts upon trehalose, a glucose dimer with 1-1 carbon linkage which is not highly significant in human consumption.

The combined hydrolytic activity of the brush border enzymes results in the release of free monosaccharides which are subsequently absorbed by various carrier systems or passive diffusion (see next section). The main products of the final digestive step include glucose and lesser amounts of fructose and galactose. The brush border hydrolysis step is rapid and efficient with the exception of lactose hydrolysis by lactase. Therefore, monosaccharides may accumulate in small amounts during the digestive process. Thus, the rate-limiting step to the assimilation of carbohydrate by the body is likely the transport process from lumen to enterocyte.

1.6.4) UPTAKE OF MONOSACCHARIDES

As with other nutrients, the absorption of monosaccharides involves functional events at both the BBM and BLM. In the case of hexose absorption asymmetric transport

mechanisms play an important role in accomplishing the net transport. In addition, the presence of the UWL has an influence as discussed previously (section 1.3.5).

The three major monosaccharide products of digestion including glucose, galactose and fructose are all absorbed at rates that appear to exceed passive movement. In fact, saturable, carrier-mediated transport has been observed for each monosaccharide. In addition, other characteristics of carrier-mediated transport including competitive interactions and specific inhibition have been described. Thus, monosaccharide transport has been characterized by Michaelis-Menton kinetics, as described below. As well, permeability coefficients are important. For example, as suggested earlier, there may be a significant passive component of glucose uptake in addition to the carrier-mediated movement (Pappenheimer, 1990). Thus, kinetics studies of glucose absorption in vivo are characterized by a curvilinear relationship in which, superimposed on the curve of active transport, there is a linear passive transport component (Debnam and Levin, 1975).

1.6.5) BRUSH BORDER MEMBRANE TRANSPORT MECHANISMS

Active glucose transport has been widely studied. Earlier studies by Crane et al (1962) suggested that carrier-mediated glucose transport was a Na-dependent process. These authors postulated that the flow of Na in a chemically favourable direction across the plasma BBM provided the energy for the accumulation of hexose against its concentration gradient. Furthermore, early studies with unidirectional flux chambers suggested that the influx of sugar was determined primarily by the extracellular concentration of Na and was a saturable function of either Na or sugar concentration in the bathing solution (Goldner et al, 1969). In subsequent years, studies with intact tissue, isolated cells and more recently membrane vesicles, have confirmed the involvement of Na in the transport process. An imposed Na gradient will induce a sugar gradient in each experimental situation.

However, continued studies also indicated that the chemical gradient for Na would not provide adequate energy to accomplish the observed rates of glucose transport (Kimmich, 1981). It is now recognized that membrane electrical potentials are also an important factor in the bioenergetics of glucose/hexose transport in addition to the Na gradient of chemical potential (Kimmich, 1981). The importance of membrane potential has been demonstrated by the use of valinomycin which increases membrane permeability to K. When a Na gradient is imposed simultaneously with valinomycin, the induced substrate uptake is several-fold greater than with Na gradients alone (Murer and Hopfer, 1974). Furthermore, the carrier movement of glucose is electrogenic; it is well established that active transport of glucose induces a significant and rapid depolarization of the electrical potential difference across the BBM which cannot be accounted for by intracellular Na levels alone (Rose and Schultz, 1971).

The inward movement of Na is favoured by the Na-K-ATPase in the BLM which maintains electrochemical gradients for Na and K. The importance of this mechanism is confirmed by the use of the glycoside ouabain which inhibits the action of the Na pump and simultaneously inhibits hexose-stimulated Na absorption and electrical currents across the epithelium (Schultz and Zalusky, 1964). Thus, although the glucose carrier itself is not directly energy dependent, metabolic activity or ATP expenditure is required for the activity of the Na pump to maintain the transmembrane potential difference. The presence of Ca dependent K channels (CDPCs) has been reported in the BLM of rabbit (Brown and Sepulveda, 1985) and chick enterocyte (Montero et al, 1990). Enhanced K permeability (via these channels) associated with Na-dependent substrate transport may also be important in energizing the movement of hexoses across the BBM.

The nature of the relationship between Na and the glucose carrier is not completely delineated. Recent studies suggest that the Na effect occurs at the level of the BBM and may be variable between species. For example, in rat, hamster, guinea pig and human intestine, Na may modify the affinity of the carrier for glucose but not the maximal transport rate; whereas the opposite situation may be characteristic of rabbit and chick (Kimmich, 1981; Thomson, 1983). In a study, correcting for the effects of the UWL as well as concomitant passive glucose permeation, Thomson (1983) studied the effects of Na on the kinetic parameters of in vitro glucose uptake into rabbit jejunum. The author reported that both affinity and velocity components of the carrier were influenced by the concentration of Na in the external environment. Furthermore, the passive permeability of the tissue to glucose was increased with increasing concentrations of Na. These observations have important implications in defining the kinetic model of the glucose transporter and the order of substrate and cosubstrate binding patterns.

Several studies have assumed a stoichiometry of 1 Na ion for each glucose molecule transported by the carrier across the membrane. However, other workers have suggested that the ratio is more likely 2:1 for Na to glucose (Kessler and Semenza, 1983; Kimmich and Randles, 1980; Kimmich, 1981). These authors propose that since basal entry of Na is a potential-dependent event, the addition of sugar (which depolarizes the BBM) diminishes Na entry by routes other than the sugar-induced movement. The net result is presumably lower basal fluxes. Since, sugar-induced Na entry is calculated by subtracting the stimulated from basal values, it is possible that the assumed basal value is higher than the true value, thus resulting in erroneous stoichiometry calculations. In isolated chick enterocytes with experimentally controlled membrane potential and monovalent ion transport, stoichiometries of 2:1 have been measured (Kimmich and Randles, 1980).

Stoichiometries of greater than 1:1 have been suggested in rabbit and bovine intestinal vesicles (Kaunitz and Wright, 1984). Using a method incorporating controlled vesicular glucose and Na gradients (Freeman et al, 1987) have suggested stoichiometries of greater than unity in rat intestinal tissue. The stoichiometry of Na:glucose has important energetic implications, since the free energy released as an ion flows down a gradient of electrical potential is an exponential function of the number of ions transferred (Kimmich, 1990).

The reports of varying stoichiometries, among other experimental observations, have led to the speculation that in some species there may be two distinct, simultaneously functioning, Na-dependent glucose transporters (Malo, 1988). Two transporters have been described in kidney membranes including a high capacity, low affinity system (in cortical BBM) and a low capacity, high affinity system (in outer medullary membranes) (Turner and Moran, 1982). Kinetic analyses of intestinal tissue as well as varying substrate specificities, and indirect evidence suggesting a sigmoidal relationship between glucose flux and experimental extracellular Na concentration, have all been suggestive of two carriers with different affinities in the intestine (Hopfer, 1987). However, the evidence for two transporters remains inconclusive and inconsistencies have been reported. It has been suggested that in mammals, the major glucose transporter exhibits a 1:1 Na to glucose stoichiometry and there may be a possible second transporter with higher ratios that vary with species, age, intestinal region and experimental preparation. Kimmich (1990) suggests that a single transport system may provide kinetic data that may be interpreted as two or more carrier systems, particularly if membrane potential is not controlled. Furthermore, Wright and coworkers have recently cloned a glucose transporter (Ikeda et al, 1989). Their cloning studies suggest that if there are other Na-dependent hexose transporters in rabbit

intestine they are not homologous to their reported single cloned transporter. Clearly, more research is needed.

As with other carriers, the glucose transporter(s) demonstrate substrate specificity and preferred structural requirements. In particular, the orientation of the hydroxyl group at the second carbon of the pyranose ring appears important for the Na-dependent transport mechanism, although there is flexibility in the structure. Inhibition by structurally similar substrates has been reported. Phlorizin is a β-glucoside that binds to the glucose site on the transporter in a Na and membrane potential-dependent manner. Thus, this competitive inhibitor of Na-coupled glucose transport has been widely used to measure the number of transport sites and characterize the system. These types of studies have suggested that under normal circumstances the majority of active glucose transport occurs in the upper one-third of the villus (Chang et al, 1987). However, it is possible under certain conditions that the distribution of transport sites may be altered (see section 1.7).

D-glucose uptake is inhibited by D-galactose at the BBM and it is currently thought that glucose and galactose are transported by the same carrier. However, this has been challenged by several authors. Inhibition studies with uranyl nitrate showed suppression of galactose but not glucose transport in vivo and in vitro (Newey, 1966). Differential patterns of inhibition of glucose and galactose by phlorizin have been reported (McMichael, 1973). Thomson et al (1987a) studied the in vitro interactions between glucose, galactose and 3-0-methylglucose in conditions of reduced UWL resistance. Based on in vitro uptake patterns, these authors determined that single Michaelis-Menton functions or single Michaelis-Menton plus competition functions could not explain the observed results. They postulated that multiple carriers might be involved including a possible carrier with common competence for all three study probes, and a second carrier with preferential galactose

transport capacity (Thomson et al, 1987a). They proposed an interesting speculation that alternatively, a single carrier exhibiting varying transport capacities during enterocyte maturation and migration might account for the observed properties.

D-glucose uptake is not inhibited by L-glucose. L-glucose does not appear to be transported by the glucose carrier except at extremely low concentrations. Thus, the L-isomer may be used experimentally as an indication of passive glucose permeation in the intestine.

Fructose transport appears to occur by a mechanism independent of the Na-mediated transport of glucose and galactose. In vivo studies have suggested that fructose absorption is not due to simple diffusion but occurs via a specific mechanism that is independent of glucose transport (Esposito, 1984).

The biochemistry of the glucose transporter is currently being defined. However, until it is isolated and purified, many of the above questions regarding stoichiometry, multiplicity of carriers etc. will remain speculative.

1.6.6) KINETICS OF TRANSPORT AND THE PASSIVE COMPONENT

Kinetic studies have suggested that the affinity constant (Km) for the intestinal transport of sugars ranges from 5-10 mM in intact tissue (Alpers, 1987). However, since many studies have not corrected for UWL effects, it is likely the true Km are considerably lower with estimates of 0.5 to 1.9 mM reported. Studies in isolated BBM have ranged from 0.2 to 4 mM in so-called "low-affinity systems" and less than 50 μm for "high affinity systems" which vary between species (Hopfer, 1987). As discussed previously, the concentration of Na will influence the kinetic parameters. In rabbit jejunum, the true Km for glucose uptake was 3 mM in the presence of 25 equivalents/L Na but decreased to 0.7 mM as the buffer Na concentration was increased to 75 equivalents/L (Thomson, 1983).

Following the ingestion of a carbohydrate meal it is clear that glucose concentrations in the lumen will greatly exceed these values and thus as much as 80% of glucose transport will occur under conditions of a favourable chemical gradient for this hexose (Alpers, 1987). It has been suggested that this favourable transport may utilize the same carrier system as the active transport mechanism but without energy expenditure. The passive component is likely considerable under these conditions as well and may account for more than half of glucose uptake under physiological conditions (Pappenheimer, 1990).

However, at low concentrations (1 mM) of 3-0-methylglucose in isolated chick cells and confirmed in vesicle work, approximately 90% of the total influx of this hexose was via the Na-dependent, phlorizin sensitive pathway. Thus, the active transport mechanism is important in mediating uptake when the chemical gradient is dissipated and luminal concentrations become low. In addition, since nutrient backflux into the lumen is occurring continuously (see below) the active transport provides a mechanism for continual reabsorption of the nutrients from the UWL (Kimmich, 1990).

1.6.7) BASOLATERAL TRANSPORT MECHANISMS

As suggested, the net movement of hexoses must involve transport processes in both membranes. Experimental evidence in isolated cells and vesicles suggests that the exit of glucose from the cell across the BLM is mediated by a carrier that is independent of Na and does not actively transport substrates (i.e. a facilitated diffusion process). The carrier demonstrates saturation kinetics, and different substrate specificities and structural requirements than the BBM carrier. However, there is some overlap in function; both the BBM and BLM mechanisms are able to transport both D-glucose and galactose. The BLM is commonly characterized by its sensitivity to phloretin and other structural analogues of flavones and flavonones as well as cytochalasin B.

As a mechanism of glucose influx, serosal transport mechanisms do not contribute greatly. As indicated above in isolated intestinal cells, at low concentrations of 3-0-methylglucose, the great majority of cellular influx occurs via the BBM carrier; approximately 7% may be mediated by the serosal component and 3% by passive unidirectional flux (Kimmich, 1981). However, Kimmich suggests that as sugar accumulates to a steady state in the cell, the facilitated diffusion and nonmediated diffusion pathway account for approximately 90% of the sugar movement from the cell (Kimmich, 1990). The remaining portion may involve efflux into the lumen via the BBM carrier (Kimmich, 1981). It is also suggested that serosal transport processes have a significant impact on the developed gradients of sugar due to the dissipation of these substrates through the efflux pathways. In this regard, inhibition of BLM transport with the aforementioned inhibitors results in extremely high sugar gradients across the cell. Thus, the potential thermodynamic capability of the glucose transport system appears to far exceed that normally observed under physiological conditions.

1.7) GASTROINTESTINAL ADAPTATION

There is abundant evidence to suggest that the nutrient transport processes of the small intestine adapt to a variety of physiological, pathological and environmental stimuli. Functional and morphological alterations have been observed in response to dietary manipulation, starvation, hyperphagia, gestation, lactation, aging, ethanol ingestion, drug intake, diabetes, abdominal irradiation and intestinal resection (Thomson et al, 1989c; Thomson et al, 1990). Although it is not the intent of this review to provide details of each phenomenon, several characteristics of these adaptive responses will be considered in the context of mechanisms of adaptation.

1.7.1) MECHANISMS OF ADAPTATION

A) Morphology

One of the simplest mechanisms of adaptation in response to a given stimulus is an alteration in the intestinal surface area available for absorption. This may occur at the macroscopic level by increasing the length and diameter of the bowel. For example, following intestinal resection, increases in these parameters have been reported in the residual bowl of humans (Postuma et al, 1983) dogs (Shin et al, 1980) and pigs (Sigalet et al, 1990).

Microscopically, alterations in villus height, width and density may occur, thus altering the mucosal surface area available for nutrient transport. This phenomenon has been well characterized as a process of intestinal adaptation during lactation and in the remnant bowel following intestinal resection. Karasov and Diamond (1987) suggest that these responses may be classified as "nonspecific adaptation" in which absorption of several nutrients is simultaneously increased by the primary mechanism of increased absorptive area. Hyperphagia is associated with a similar response and in fact may mediate the adaptation associated with lactation in experimental animals. In some species, hypertrophy of the individual villi may occur while there is a decrease in the actual number or "density" of villi (Sigalet et al, 1990); the overall result however, may be an increased or unaltered absorptive surface area.

In many cases, alterations in intestinal morphological parameters do not entirely account for the direction and magnitude of the observed functional responses (Keelan et al, 1985a, 1985b; Thomson et al, 1989c). Thus, alterations in nutrient transport need not be mediated solely by changes in total mucosal surface area. This is likely because the functional surface area of the villus may not be the same for all nutrients. As described

below, the upper portion of the villus is important for the transport of glucose and amino acids (Chang et al, 1987; Maenz and Cheeseman, 1986). Furthermore, the portion of the membrane surface area used for the passive uptake of different lipids may also be variable (Winne, 1978). Therefore, static measurements of mucosal surface area do not necessarily indicate which functional portion of the villous may be affected by various stimuli (Winne, 1978; Thomson et al, 1989b, 1989c).

B) Cell Kinetics and Distribution

Under normal circumstances there is an equilibrium between cell production and division in the crypt and cell loss at the tip of the villi (Williamson, 1978). As enterocytes migrate up the villus from the crypts they mature and gain the ability to transport various substrates. The adaptive response may influence both the rate at which enterocytes are produced and released from the crypt as well as the time required to migrate from the crypt to the villus tip, and finally the rate of turnover. For example, hyperplasia in both the mucosal and crypt cell compartments has been observed in association with lactation and intestinal resection (Johnson, 1987). Increased cell migration rates have been observed, although overall cell turnover may not change due to elongated villi (Williamson, 1978).

Furthermore, the signal along the villus for initiating the transport function may be subject to adaptive regulation (Cheeseman, 1986; Thomson et al, 1989b). The age of the cell, in addition to the position on the villus are likely both important (Thomson et al, 1989c). As indicated earlier, under normal circumstances the upper portion of the villus appears to be utilized for nutrient transport (Chang et al, 1987; Maenz and Cheeseman, 1986; Cheeseman, 1986). However, in the early post-irradiation phase, microdensitometry techniques revealed that enterocytes express the ability to transport amino acids at an earlier age and at a position closer to the base of the villus than the control animals (Thomson et

al, 1989b). By 7 days post-irradiation, the transport and migration properties return to normal. Alterations in the distribution of transporting enterocytes along the villus may also be an important adaptive response for glucose transport in experimentally-induced diabetes (Chang et al, 1987; Fedorak, 1990).

C) Unstirred Water Layer (UWL) Effects

As indicated earlier (section 1.3.5), the UWL adjacent to the cell membrane may have important effects on the uptake of nutrients. Therefore, alterations in this barrier may play a role in the adaptive process. Several phenomena have been associated with changes in the effective resistance of the UWL including dietary manipulation, aging and diabetes (Thomson, 1984). However, changes in the UWL do not always account for the direction and magnitude of the observed nutrient transport changes.

D) Brush Border Membrane Composition

The fluidity and passive permeability characteristics of BBM and all membranes is influenced by the relative content of cholesterol, phospholipid, the nature of the fatty acid composition of the PL and the presence of proteins. Altering the lipid fluidity may affect the immediate environment of integral digestive and transport enzymes or the bulk lipids in the membrane. Thus, factors that alter lipid content or distribution may influence transport function as well as permeability of membranes.

Changes in intestinal transport function have been described in association with altered BBM composition (Keelan, 1985a, 1985b, 1985c; Thomson and Keelan, 1985; Brasitus and Schacter, 1982; Meddings et al, 1990). The altered lipid composition in these studies was induced by aging, fasting, diabetes and dietary manipulation.

Altering the fatty acid composition of the diet has been well studied. Dietary differences in fatty acid intake alter fatty acyl tail composition of PL in plasma membranes

of rat intestinal mucosa, as well as liver and brain (Clandinin et al, 1983, 1985). Brasitus et al (1985) demonstrated that altering the composition of fatty acid components in TG had a significant influence on cholesterol content, cholesterol:PL molar ratio, the saturation of acyl chains and the membrane fluidity of rat BBM.

Furthermore, the fatty acid composition of the diet may play a role in modifying the functional adaptive response to other stimulating effects. For example in irradiated rats, a diet high in saturated fatty acids prevented radiation-induced alterations in the uptake of several lipids and glucose whereas an isocaloric diet high in polyunsaturated fat did not (Thomson et al, 1989b). In experimental diabetes (streptozotocin-induced) a high polyunsaturated diet, initiated before streptozotocin treatment and hyperglycemia, prevents the characteristic induction of glucose and lipid uptake in these animals (Thomson et al, 1987b). Feeding animals a diet enriched with polyunsaturated fatty acids reduces glucose and galactose uptake compared with animals on a diet high in saturated fatty acids (Thomson, 1987).

At the intracellular level it is probable that alterations in the enzymes of lipid metabolism (e.g. desaturase acylation and deacylation enzymes) play a role in altering the membrane lipid composition. Desaturase enzymes with the ability to desaturate and elongate essential and nonessential fatty acid have been demonstrated in the enterocyte, thus implying that these enzymes are important for the synthesis of long chain polyunsaturated fatty acids in the enterocyte membrane PL (Garg et al, 1988). It is postulated that external and internal stimuli can alter the activity of fatty acyl desaturase with resulting alterations in membrane PL composition and possible further changes in cholesterol and/or PL synthesis. The resulting alterations in the physicochemical properties of the membrane may then account

for altered permeability and transport characteristics which represent the adaptive response to the stimulus (Garg et al, 1990).

E) Transport Kinetics

Alterations in specific transport mechanisms required for active transport and facilitated diffusion may play a role in the adaptive response. There have been reports of altered maximal transport capacity (Jmax), and affinity constants (Km) as well as altered passive permeability characteristics of nutrients.

The major adaptive alteration in carrier-mediated transport is likely a change in the Jmax versus alterations in carrier affinity (Karasov and Diamond, 1983). Increased mucosal surface area and a corresponding increase in the total number of enterocytes is the simplest nonspecific means of increasing total transport capacity. However, an increased Jmax has been observed in the absence of alterations to mucosal surface area. Substrate-induced synthesis of carriers without alterations in mucosal surface area has been proposed as a mechanism for the increased maximal transport capacity of the jejunum in response to a high carbohydrate diet. This type of effect may also account for the maintenance of the observed proximal to distal gradient of glucose transport in the gastrointestinal tract of most species.

Alternatively, the total number of transporting enterocytes may be altered without a change in the total number of enterocytes. Altered distribution of transporting enterocytes has been reported as an adaptive mechanism for both glucose (Chang et al, 1987; Fedorak, 1990) and some amino acids (Cheeseman, 1986).

In the case of Na-coupled transport processes it is also possible that nonspecific mechanisms such as altered electrochemical gradients might be expected to influence maximal transport capacities for some nutrients. However, this has been ruled out in many

cases, due to dissimilar effects on several Na-coupled nutrients (Karasov and Diamond, 1983, 1987).

Alterations in the conformation or biochemical state of a carrier might be affected to influence the affinity of the carrier for its substrate (Km). As indicated above, this is a less commonly reported adaptive phenomenon, although it cannot be ruled out as potential mechanism of intestinal adaptation (Thomson, 1984).

Alterations in the passive permeability to nutrient transport may be affected by the composition of the intestinal membranes as discussed above. Nutrients may also pass through the paracellular route via tight junctions driven by electrochemical potential differences and by the solvent drag exerted by water flux (Pappenheimer, 1990). Alterations to the tight junctions may therefore affect nutrient uptake although this is not widely reported as an adaptive effect in the intestine.

1.7.2) SIGNALS FOR ADAPTATION

A) Luminal Factors

The presence of food in general, as well as specific nutrients in the diet have both direct and indirect effects on the form and function of the intestine (Thomson and Keelan, 1985).

The importance of the general presence of luminal nutrients in the intestine is demonstrated by the observation that mucosal atrophy and reduced transport function are associated with parenteral feeding (Lo and Walker, 1989). Furthermore, the provision of oral nutrition appears to be a major factor in optimizing the adaptive response of the remnant bowel after resection. In fact, pairfeeding studies suggest that hyperphagia may play a role in the genesis of adaptation in intestinal resection and lactation (Williamson, 1978).

Hyperphagia associated with thermal reduction and thyroidectomy also elicits an adaptive response.

The effects of starvation also suggest the importance of the nonspecific effects of food although reports of adaptive responses in starvation are inconsistent. The consequences of short term starvation on transport appear to be similar to the dietary removal of substrate (i.e. reduced transport function). However, interpretation of data from longer starvation periods must consider potential alterations in metabolism of transported nutrients and probable thinning of the intestinal wall (Karasov and Diamond, 1983).

Active absorption or secretion stimulated by the presence of nutrients is likely more important in mediating trophic effects on the gut than the actual metabolism of the nutrients. For example, infusion of poorly metabolized but transported substrates (e.g. galactose, methylglucoside) have been shown to stimulate mucosal cell production to the same extent as glucose (Clarke, 1977). The author proposes that "functional workload" is the important factor in stimulating mucosal growth. In contrast, dietary amines have been suggested to have a direct trophic affect on the normal and adapting gastrointestinal mucosa (Johnson, 1987). Dietary fibre also stimulates mucosal growth likely mediated by decreasing luminal pH secondary to fermentation processes.

The presence of food in the gut also elicits a number of secondary responses including the secretion of pancreaticobiliary juices which themselves exert trophic effects on the mucosa. In addition, the ingestion of food initiates the secretion of peptides into the lumen. Epidermal growth factor has been shown to stimulate growth intraluminally. Gastrin is also an important factor affecting the growth of gastrointestinal mucosa (Johnson, 1987).

In summary, the general presence of food initiates intestinal secretion, absorption and motility as well as stimulating nerves and the release of trophic hormones. Thus, it is difficult

to separate the direct effects from the indirect influence of food in the intestinal lumen (Thomson et al, 1989c).

In addition to nonspecific effects, there appears to be a relationship between the dietary content of some specific nutrients and the uptake of the same. In general, transport of nutrients that are considered "nonessential and/or used for calories" (e.g. carbohydrates) appear to be upregulated in response to increased dietary intake of the specific substrate (Ferraris and Diamond, 1989). The relationship between dietary protein content and uptake is more complex due to the essential nature of some of the amino acids and the multiple carriers involved, including acidic, basic and neutral transporters and peptide carriers. To study amino acid effects Karasov et al (1987) used low nitrogen rations with essential amino acids in rats to avoid the deterioration in intestinal mass and structure that occurs with traditional low protein diets. They demonstrated that the transport of nonessential amino acids (e.g. proline, aspartate) increased in relation to the dietary protein level whereas the response of essential amino acids was more variable. Low concentrations of essential amino acids appeared to downregulate uptake whereas with higher amounts upregulation occurred but to a lesser degree than that observed for nonessential protein.

For many minerals, including iron, Ca, zinc and phosphate, transport is downregulated in response to a high dietary content of each. This response appears to be characteristic of essential nutrients that do not yield calories or that may be toxic if excessive absorption occurs (Ferraris and Diamond, 1989).

The signals for specific transport are not always as clear cut as in the case of fructose and aspartate which appear to upregulate their own carriers specifically. For example non-transported substrates such as mannose and fructose appear to exert the same regulatory and induction effects as glucose does, whereas the nonmetabolized but transported glucose

analogue 3-0-methyl glucose does not (Ferraris and Diamond, 1989). A similar paradox has been reported for amino acid and peptide transporters.

B) Distal Signals

In addition to luminal factors, experimental evidence suggests that circulating humoral factors may be associated with the adaptive response of the intestine. Enteroglucagon and epidermal growth factor may be important in mediating the effects (Thomson et al, 1989c). For example, following partial intestinal resection, plasma enteroglucagon levels increase significantly. Other potential humoral agents have been suggested but there is no conclusive evidence for a specific substance at this point.

1.7.3) POLYAMINES AND INTESTINAL ADAPTATION: A ROLE FOR CALCIUM?

An increase in the polyamines putrescine, spermidine and spermine and their rate-regulating synthetic enzyme ornithine decarboxylase (ODC) is one of the earliest events in cell growth, replication and differentiation (Koenig et al, 1983). The polyamines may be an important factor in the adaptive response of the intestine, particularly in association with mucosal growth. The activity of ODC in the mucosa of rat small intestine is elevated after feeding, partial resection, during lactation, and in other experimental manipulations associated with enhanced mucosal growth (Luk and Yang, 1987; Hosimi et al, 1987). Inhibiting ODC activity with a specific inhibitor (DL-α-difluoromethylornithine or DFMO) prevents the usual mucosal growth associated with the adaptive responses and prevents the accumulation of polyamines. Thus, it is apparent that polyamines somehow mediate some of the observed intestinal adaptive responses. Their exact function at the molecular level has not been determined; direct effects on RNA activity have been postulated.

The induction of ODC in several cultured cell types by various stimuli appears to be mediated by Ca and/or cyclic adenosine monophosphate (cAMP). For example, in an

osteogenic sarcoma cell line (UMR 106-01) forskolin and parathyroid hormone stimulated ODC activity was associated with a significant increase in intracellular Ca (Van Leeuwen et al, 1988). Furthermore, the addition of verapamil to the cellular media dose-dependently decreased the stimulated, as well as basal ODC activity. A role for extracellular Ca is strengthened by the observation that EGTA and $LaCl_3$ had similar effects. These results were independent of effects on cAMP production; in fact, cAMP production was enhanced by these agents. In contrast however, Ca ionophores could not induce basal or stimulated ODC activities in these cells. The authors concluded that a rise in intracellular Ca levels without a rise in cAMP is not sufficient to stimulate activity and thus both mediators are likely involved in polyamine induction.

The presence of extracellular Ca has been associated with ODC stimulation in other in vitro cell cultures as well (Langdon, 1984). However, in cultured keratinocytes Ca stimulation of ODC activity was only demonstrable in cells that were previously Ca deprived. The stimulation was proportional to the duration and degree of Ca deprivation.

In kidney cortex, it was shown that stimulation of membrane transport processes (testosterone-mediated) was associated with increased polyamine synthesis. The stimulation also increased Ca fluxes and mobilization from mitochondria, resulting in increased cytosolic Ca concentrations (Koenig et al, 1983). Each of these processes could be blocked by DFMO.

Whether Ca is involved in the polyamine synthesis of intestinal tissue is unknown. It has been observed that ODC levels are higher in villus than in crypt cells, thus suggesting potential importance in mature or differentiating cells (Baylin et al, 1978; Sepulveda et al, 1982). However, indirect evidence has suggested that intracellular Ca may not be involved;

agents such as gastrin and CCK that alter Ca levels in intestinal the cells do not appear to affect the activity of intestinal ODC (Johnson, 1987).

In addition to it's potential association with the polyamines, Ca is well recognized as a direct regulator of cell growth. Ca has been shown to be important from the G1 to the S phase in DNA replication. Furthermore, several reports suggest a role for Ca and calmodulin (CAM) in mitosis (Rasmussen and Means, 1989).

Thus, it is likely that Ca may be involved in the adaptive processes occurring in the intestine. Whether modifications in cellular influx via CCBs might somehow alter intestinal adaptation directly or via polyamine effects remains to be determined.

1.8) CALCIUM

1.8.1) INTRODUCTION

In 1883, Sydney Ringer discovered that Ca was essential for the contraction of cardiac muscle. Subsequent years of work by numerous investigators have shown that Ca not only mediates the contraction of all forms of muscle but is also involved with many other cellular processes. In fact, Ca is the critical component in the so called "Ca messenger system". This nearly universal message system regulates cell function by translating or "coupling" events at the external cell surface (i.e. stimuli) to responses in the cell. Examples of Ca regulated processes include the secretion of exocrine, endocrine and neurocrine products, the metabolic processes of glycogenolysis and glyconeogenesis, the transport and secretion of fluid and electrolytes and the growth of cells (Rasmussen, 1986a).

Under resting conditions Ca is present in concentrations of approximately 1000 μm outside of the cell. Intracellular concentrations in a variety of cell types have been reported and range from 0.05 to 0.35 μm, although the most commonly reported value is approximately

0.1 μm (Carafoli, 1984; Rasmussen and Barrett, 1984). Thus, there is a 10,000-fold gradient of Ca across the plasma membrane of most cells studied. The coordination of the complex system involved in maintaining this gradient will be discussed briefly in the following section.

Stimulation of the cell by an appropriate messenger generally causes a rise in the intracellular concentration of Ca by an estimated 0.65 to 1.5 μm (Rasmussen and Barrett, 1984). Alterations in cytosolic Ca concentration (in both directions) may occur by a variety of mechanisms as outlined below. Very small changes in the concentration of Ca in the cytosol initiate a series of effects that ultimately regulate the function of the cell. The mechanisms of this process are discussed in more detail (see Calcium Messenger System).

Although the basic aspects of cellular Ca metabolism are similar in most mammalian cells, each cell type has its own particular specialization of the components involved. Thus, the means of translating small changes in intracellular Ca concentration into cellular responses may be varied and complex. A general summary of these processes is provided below.

1.8.2) THE CALCIUM MESSENGER SYSTEM

The transmission of information from an external cell surface receptor to the cell interior by the Ca messenger system appears to involve two operationally and temporally distinct branches. The proposal of a general sequence of events was developed by the work of Rasmussen and colleagues (1984, 1986b). These authors used a highly specialized endocrine cell, the adrenal glomerulosa, that produces a single product. The observations in this system have been based largely on the stimulation of the cell by angiotensin II to induce the production of the steroid hormone aldosterone. However, similar general events have been observed in a variety of other cell systems. Thus, the model proposed by these

authors may represent a general mechanism by which the Ca messenger system operates to initiate and sustain cellular responses to external stimuli.

The first branch of the proposed Ca messenger system has been designated the calmodulin "(CAM)-branch" and appears to mediate the initial cell response to stimulus. The second branch has been termed the "protein C-kinase branch" and is responsible for the sustained phase of cellular response. The two branches are depicted in figure 1-1.

A) Initial Response - Calmodulin Branch

The initial sequence of events occurring after a hormone interacts with its receptor is not completely defined. However, the general effect involves the activation of a specific phospholipase, called phospholipase C. This enzyme in turn catalyzes the hydrolysis of a phosphoinositide that is present in the plasma membrane, phosphatidyl-inositol 4,5-biphosphate (PdtIns[4,5]P_2). This hydrolytic action generates two important cellular messengers; these include inositol 1,4,5-triphosphate (Ins[1,4,5]P_3) and diacylglycerol (DG), the latter of which is rich in arachidonic acid (thus providing a potential source of arachidonic acid for the production of leukotrienes, prostaglandins, prostacyclins and thromboxane synthesis) (Berridge, 1984, 1989; Rasmussen and Barrett, 1984; Rasmussen, 1986b).

The Ins(1,4,5)P_3 is water-soluble and thus diffuses into the cytosol of the cell where it acts to mobilize Ca from internal stores. The Ca mobilizing activity of this messenger was first demonstrated in pancreatic acinar cells and has subsequently been observed in a variety of cell types (Streb et al, 1983). Although the site and mode of action of intracellular Ca release are still being investigated, it appears that a particular Ins(1,4,5)P_3-sensitive component of the ER is the likely site. It has been proposed that Ins(1,4,5)P_3 binds to a receptor which then activates a channel permitting the release of Ca into the cytosol.

Simultaneous with the increased release of Ca from an inner pool, an enhanced influx of Ca from the external environment appears to occur. The influx is likely mediated via receptor operated Ca channels (ROCC) and/or voltage dependent Ca channels (VDCC) in the plasma membrane. The combined results of intracellular mobilization and extracellular influx result in a sharp rise in the cytosolic content of Ca.

In the cell, Ca may interact directly with enzymes to modulate their function. However, in many cases the Ca interacts with a universally distributed Ca receptor protein called CAM. CAM binds Ca to at least three of four potential binding sites thus inducing a conformational change and the formation of a Ca-CAM complex. This complex can then act either directly with proteins/enzymes to elicit a response or may act indirectly to regulate activity via Ca-CAM dependent phosphorylation of appropriate enzymes.

There are a number of important kinases considered to be Ca-activated and CAM-dependent. Examples include phosphorylase b kinase, myosin light chain kinase, and general Ca-CAM-dependent kinase. Kinases catalyze the ATP-dependent phosphorylation of specific substrate proteins (enzymes), altering their structural and kinetic properties. Thus, they are highly important in cell function and regulation.

An example of a Ca-CAM directed phosphorylation occurs in hepatic glycogenolysis. For example, phosphorylase b kinase is activated by Ca-CAM to catalyze the phosphorylation of the enzyme phosphorylase thus converting it from its inactive to active form. Recently, a Ca-CAM dependent kinase has been suggested to play a role in the short-term regulation (via phosphorylation and concomitant inactivation) of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMG-CoA reductase), the rate-limiting enzyme for cholesterol synthesis (Beg et al, 1987a, 1987b). Recent studies in the small intestine suggest that Ca-CAM dependent phosphorylation reactions (via kinases in the BBM) may be important in the regulation of

intestinal ion transport. These are discussed further in the section on "Mechanisms of Calcium Regulation". It is not certain whether CCBs influence these kinase-mediated phosphorylation reactions.

An important example of direct interaction with Ca-CAM in most cells is the activation a Ca pump (Ca-ATPase) in the plasma membrane by increased concentrations of Ca-CAM in the cytosol. As a result of this pump activity and the sequestering of large amounts of Ca into a nonionic mitochondrial Ca pool (both described further below), the stimulated elevated Ca level in the cell returns to basal levels (or just higher than basal). For example, in glomerulosa cells stimulated by Angiotensin II, a transient rise in cytosolic Ca peaks at 1 minute and returns to nearly basal values within 2 to 4 minutes (Rasmussen and Barrett, 1984).

However, in spite of the decline in intracellular Ca levels the elicited response (i.e. aldosterone secretion) is initiated in 5 to 8 minutes and slowly increases to a sustained value by 15-20 minutes. Furthermore, although cytosolic levels return to baseline, the influx of Ca across the plasma membrane remains elevated and appears to be balanced by an elevated rate of efflux. The mechanisms responsible for the sustained influx of extracellular Ca are poorly understood. It has been speculated but not confirmed that the hydrolytic activity of phospholipase C may alter membrane structure and thus Ca permeability. Alternatively, $Ins(1,4,5)P_3$ itself or products of arachidonic acid metabolism could play a role.

B) Sustained Response - Protein Kinase C Branch

The observation that the intracellular Ca concentration returns to basal levels even in the presence of continued stimuli (and cell response) led to the speculation that a second branch of the Ca messenger system must be active in sustained responses. This branch has been designated the protein kinase C branch and is related to the second product formed

by the initial hydrolysis of PtdIns(4,5)P_2 which is DG. The DG remains in the plane of the membrane and plays and important role in activating a kinase designated as protein kinase C (Takai et al, 1979).

Protein kinase C is present in the free form in the cytosol of most cells and in the resting state is relatively insensitive to Ca. The interaction of this kinase with the increased DG content of the plasma membrane in the activated cell increases its sensitivity to Ca and also promotes its binding to the plasma membrane. The presence of PL in the membrane (particularly phosphatidylethanolamine) further enhances its sensitivity to Ca. Hence, this kinase has been described as a Ca-activated, phospholipid-dependent protein kinase.

In summary, it is likely that the CAM branch and the protein kinase C branch function either sequentially in some cell types (perhaps the intestine as will be discussed) or synergistically in others (e.g. adrenal glomerulosa) to initiate and maintain a cellular response via the Ca messenger system.

1.8.3) CALCIUM INFLUX AND EFFLUX MECHANISMS

Ca is transported into and out of cells by several mechanisms involving both the plasma membrane and the intracellular organelles. The mechanisms most relevant to the experimental work of this thesis involve Ca entry through channels in the plasma membrane. Therefore, most of the following discussion focuses upon Ca channels; other transport mechanisms will be briefly included to complete the review.

1.8.4) CALCIUM CHANNELS AND THE PLASMA MEMBRANE

One of the important characteristics of the plasma membrane is its relative impermeability to the Ca ion. This impermeability contributes to the maintenance of intracellular Ca homeostasis and the maintenance of the significant Ca gradient in the cell as mentioned previously.

As indicated above, the plasma membrane also plays an important role in mediating the response of a cell to an extracellular stimulus. This is due to the presence of Ca channels in the membrane that open in an activated cell to allow the rapid entry of Ca across the steep concentration gradient. The Ca channel may open in either a transient or sustained fashion depending upon the external signal.

In general, ion channels are proteins embedded in the lipid bilayer of the membrane. The pore or channel-forming proteins provide an aqueous route for the passive flux of ions. The movement of the charged ions into and out of the cell would otherwise be restricted through the lipid environment. Channels may be opened in response to changes in membrane potential (voltage dependent channels [VDC]) and/or by occupation of the channel-associated receptors by intracellular second messengers, neurotransmitters and/or drugs (ligand-gated or receptor-operated channels [ROC]) (Greenberg, 1987). VDC and ROC that provide a channel for the movement of Ca are referred to as voltage dependent calcium channels (VDCC) and receptor operated calcium channels (ROCC), respectively.

A) Voltage Dependent Calcium Channels

VDCC have been generally well characterized using voltage patch techniques, mainly in cardiac cells. The VDCC are closed in the unstimulated state; depolarization of the plasma membrane increases the probability of channel opening resulting in inward movement of Ca. The exact mechanisms of the voltage dependent gating are not fully understood (Reuter and Porzig, 1988).

VDCC do not display absolute selectivity; barium and strontium may move through the channel(s) in addition to Na (Tsien et al, 1987). The channels may be blocked by numerous other cations in addition to a large heterogenous group of organic pharmacologic compounds, collectively referred to as CCBs. VDCC are often characterized by their

susceptibility to blockade by various classes of these agents as outlined in the section 1.9 "Calcium Channel Blockers".

Two types of VDCC have been described in patch clamp recordings of various cardiac muscle tissues in guinea pig, dog, frog and rabbit (Bean, 1989). The VDCC subtypes differ in several respects, most notably their membrane potential gating kinetics. In general, the most common type of VDCC appears to be the L-type channel (also referred to as "slow" and "high" threshold) which is characterized by producing a long-lasting inward Ca current and requiring strong depolarizations for activity. The T-type channel (also termed "low" threshold and "fast" Ca channel) on the other hand, is associated with more transient influxes and opens at more negative membrane potentials and weaker depolarizations than the L-type channels (Tsien et al, 1987; Bean 1989). Based on preliminary electrophysiological studies, it has been suggested that L-type and possibly T-type voltage sensitive Ca channels are present in rabbit ileum (Homaidan et al, 1989).

A third type of VDCC has been described in cultured sensory neurons of chick dorsal root ganglion (Nowycky et al, 1985). Designated the N-type channels, these VDCC appear to be characteristic of sensory neurons and are only opened by a large membrane depolarization from a strongly negative potential.

The various channels also differ in their sensitivities to the organic CCBs. In general, L-type channels display a greater sensitivity to dihydropyridine structures (described below) than T-type channels. Furthermore, ionic selectivity and sensitivity to inorganic blockers also differs implying a possible different pore structure for the L and T-type channels in cardiac muscle. Similar types of Ca channels have been described in several species of mammalian arteries, veins and aortic muscle cell lines (Bean, 1989).

Traditionally, VDCC have been felt to be characteristic of excitable cells including the above. However recently, Chen et al (1988) described both L and T-type VDCC in fibroblasts, based on patch clamp recordings of whole cell and single channel currents. Rat osteoblasts also appear to have VDCC.

As indicated earlier VDCC may also be present in the BLM of rabbit ileal epithelial cells (Homaidan et al, 1989). These authors proposed two types of Ca channels (L and possibly T) based on differing magnitudes of effects and interactions with the major classes of CCBs. Further detail is provided in the section on "Calcium and Electrolyte Transport".

Thus, continuing research suggests that the presence of VDCC may be more ubiquitous then previously proposed.

B) Receptor Operated Calcium Channels

Activation of a cell surface membrane receptor with an appropriate agonist allows the entry of Ca from the extracellular environment, through ROCC. Receptor occupation by a ligand may also induce the release of Ca from an intracellular storage site or depolarize the membrane and activate the VDCC (Vanhoutte, 1988). Whether the ROCC are in fact separate structures or whether the association of ligands with VDCC alters the channel characteristics and sensitivity is controversial.

Benham and Tsien (1987) have published direct electrophysiological evidence for ROCC by observing the effects of ATP on Ca influx in vascular smooth muscle cells from rabbit ear artery. These authors concluded that ATP-activated channels were present and were different from VDCC in many respects. The ROCCs could be activated at much lower membrane potentials and exhibited less of a voltage dependent relationship. In addition the ATP-activated channels did not exhibit the same characteristics of blocking by organic

compounds or cations. Similar ROCC's activated by N-methyl-D-aspartate have been suggested in neurons (MacDermott et al, 1986).

C) Leak Channels

Divalent cation permeable leak channels were also demonstrated in the work of Benham and Tsien (1987). These channels appear to be active in the absence of depolarization or ligand. The presence of similar channels has been suggested in T-helper lymphocytes. Although not widely reported, this mode of Ca entry may affect the basal level of free Ca in some cell types.

In the intestine, a significant component of cation movement occurs through the tight junctions. It is unknown how CCBs influence the tight junction or ionic movement via this pathway. However, Ca does play a role in maintaining the integrity of the tight junction and is therefore an important influence (see section 1.10.6).

1.8.5) OTHER CALCIUM TRANSPORT MECHANISMS IN PLASMA MEMBRANES

A Ca pump located in the plasma membrane of most tissues designated Ca-adenosine-triphosphatase (Ca-ATPase) is an important mechanism for facilitating Ca movement out of the cell. This enzyme is likely significant in non-excitable cells, and has been identified in the BLM of rat duodenum (Ghijsen et al, 1982). It has a high affinity for Ca (Km less than 1 μm) and as indicated earlier, is regulated by Ca-CAM. Rising levels of Ca and CAM in the cell are thought to bind directly to the enzyme thus increasing its affinity for Ca as well as maximal transport capacity. PL may also regulate the Ca-ATPase in a similar manner.

A Na^+/Ca^{2+} exchange mechanism is also present in the plasma membrane. Although it is a lower affinity mechanism than the above Ca pump it is likely a more important mechanism in transporting Ca out of excitable cells or when large amounts of Ca need to be

removed from the cell. The enzyme stoichiometry is 3 Na ions in for each Ca ion removed from the cell; the driving force is the chemical gradient across the plasma membrane. The presence of a Na/Ca exchanger of this nature has been demonstrated in BLM from rat duodenum, jejunum and ileum (Ghijsen et al, 1983).

The effects of CCBs appear to be limited to plasma membrane Ca channel entry with no reported effects on these alternate calcium transport mechanisms. It is unknown, however, how the influence of CCBs on Na permeability and transport (section 1.10.4, 1.10.5) would influence the $Na+/Ca^{2+}$ exchange mechanism. Furthermore, if lowering intracellular calcium concentrations stimulates $Na^{+}K^{+}$ATPase activity in the BLM, CCBs may influence Na gradients and therefore possibly affect the membrane Na/Ca^{2+} exchange mechanism.

1.8.6) CALCIUM TRANSPORT MECHANISMS AND INTRACELLULAR CALCIUM LEVELS

Although this review has focused mainly on Ca transport across the plasma membrane, Ca-ATPases and exchangers are also present in the ER, sarcoplasmic reticulum (SR) and inner mitochondrial membranes of several cells. The mitochondrial membranes also contain transporters that facilitate unidirectional uptake of Ca ions.

The coordinated activity of each of the plasma membrane and intracellular Ca transport mechanisms determines the cytoplasmic concentration of this ion and thus ultimately, several facets of cell regulation. The quantitative importance of each mechanism varies from cell to cell. Generally, the Ca channels mediate rapid Ca influx, balanced by the efflux mechanisms of the Ca-ATPase. The ER and SR appear to be important for initial stimulus-induced intracellular release of Ca and for buffering small variations in cytoplasmic levels. When the capacity of the ATPase is exceeded the mitochondria actively accumulate Ca and store it in a nonionic, biologically active form. The mitochondria have been defined

as "long term" or emergency Ca buffers in the cell while the ER and SR are responsible for "fine regulation" (Carafoli, 1984).

The control of intracellular Ca levels is critical not only as a potential regulator for cell function but also because increases in the level of this ion within the cell have very toxic effects. Thus the combined goal of Ca transport mechanisms is to maintain Ca homeostasis.

1.9) CALCIUM CHANNEL BLOCKERS

1.9.1) GENERAL

A great deal of characterization of Ca channels has been based on their interaction with various agents that selectively block the movement of Ca into the cell. As indicated earlier, a large group of organic agents with "Ca blocking" activity have been described.

The designation "Ca antagonist" was coined by Albrecht Fleckenstein in 1966 (Fleckenstein, 1983). His observations were based on initial investigations of the cardiodepressant side effect of coronary vasodilators (prenylamine and, then unnamed, verapamil) in addition to several subsequent experiments. This investigator noted a specific inhibitory action against Ca currents and Ca mobilization in ventricular myocardium. The agents appeared to "mimic" Ca withdrawal. Furthermore, these effects could be overcome by the elevation of extracellular Ca concentration (Fleckenstein, 1983).

In subsequent years, numerous agents that can be classified as "Ca antagonists" have been described and designated using a variety of terms including Ca entry blockers, Ca channel modulators and CCBs used in this review. A variety of classifications have been developed to categorize this large, chemically heterogenous group of compounds. A recent World Health Organization Committee (Vanhoutte, 1987) proposed a functional classification

in an attempt to amalgamate several previous schemes. The committee's general classification of CCBs selective for Ca channels includes:

A) Type I - verapamil-like

B) Type II - nifedipine-like

C) Type III - diltiazem

Chemically, verapamil is classified as a phenylalkylamine, nifedipine is a 1,4-dihydropyridine structure (as is nisoldipine) and diltiazem is a benzothiazepine. A number of nonspecific Ca channel blockers have also been described but are not relevant to the present discussion.

1.9.2) MECHANISMS OF ACTION

Within 3 years of Fleckenstein's discovery, it was established with the use of radiolabelled [45-Ca] that the antagonist activity was a specific suppression of plasma membrane Ca movement. Furthermore, in cardiac and smooth muscle cells in which the cell membrane was removed mechanically or chemically, the usual inhibitory effects of these agents on Ca-dependent contractile activity was not observed (Rampe et al, 1985). A variety of studies confirm that of the many potential sites where Ca could be inhibited it is likely transmembrane transport that is affected by these agents (Janis and Triggle, 1983). Based on voltage-clamping experiments it has been revealed that the action of CCBs is membrane potential or "voltage" dependent. Thus, increasing inhibitory activity has been noted with decreasing membrane potential. This voltage-dependency may be more prominent in the case of verapamil and diltiazem-like agents. Thus, it has been postulated that the dihydropyridines do not need to penetrate the Ca channel to exert inhibitory effects whereas the former drugs act on the inside of the channel. However, others have observed that dihydropyridines also exhibit voltage-dependency (Carafoli, 1984). It is unknown what the

influence of varying exposure times to CCBs may have on membrane potential and/or related effects.

The molecular structure of Ca channels in plasma membranes is undetermined. Thus, it is difficult to characterize the molecular nature of the interaction between CCBs and these channels. In general however, CCBs are not believed to act as channel plugs or as simple competitors with Ca for binding. Glossman et al (1985) have proposed multiple drug receptor sites (at least 3) exhibiting allosteric regulation. For example the binding of dihydropyridines may induce a conformational change in the channel that affects the binding of other ligands (e.g. verapamil and diltiazem).

A dihydropyridine-binding peptide from skeletal muscle has recently been sequenced and the authors suggest that the receptor itself is a Ca channel. For example, purified preparations of dihydropyridine-binding proteins from skeletal muscle t-tubules have been reconstituted into lipid bilayers and function as Ca channels (Flockerzi et al, 1986).

Preliminary evidence of the molecular structure of plasma membrane Ca channels suggests that a glycoprotein of relative molecular mass 210,000 (consisting of a possible three subunits) may represent at least one of the Ca channels (Reuter, 1985).

In general, the CCB are more selective for VDCC than ROCC (Vanhoutte, 1988). For example, micromolar concentrations of the dihydropyridine nifedipine completely blocked rat aortic contraction induced by membrane (KCl evoked) depolarization. In contrast, norepinephrine-induced contraction was only reduced by 50% of maximum in the presence of nifedipine.

Various classes of CCBs also demonstrate tissue specificity. Thus, verapamil and diltiazem appear to be equally potent inhibitors in cardiac and smooth muscle cells whereas the dihydropyridines are significantly more effective Ca channel inhibitors in vascular smooth

muscle than cardiac tissue (Katz, 1986). In intestinal tissue, verapamil may be a more effective inhibitor of Ca entry than agents from the other major classes of CCBs (Homaidan et al, 1989). As described further in the context of "Mechanisms of Calcium Regulation", these authors reported that verapamil had quantitatively greater effects on ion transport in rabbit ileal tissue. Furthermore, radioligand binding studies suggested three times as many binding sites in this tissue for phenylalkylamine-derivatives (verapamil) than the other classes of CCBs.

Thus, while CCBs influence ionic transport in the ileum, a significant portion of the electrical activity of the gastrointestinal tract appears to be resistant to the activity of CCBs (Spedding, 1988). In humans, few gastrointestinal side effects have been reported in patients taking these drugs. Verapamil, however, often characterized as having more nonspecific effects than other CCBs, has been reported to cause constipation. However, CCBs may be more effective in pathological conditions in which the cells are excessively depolarized with enhanced electrical activity and resulting contractions. For example, CCBs may have a positive influence in the treatment of irritable bowel syndrome, as well as achalasia and other esophageal disorders. Further, as discussed in section 1.10.5, the use of CCBs promotes water and electrolyte absorption and therefore has the potential for clinical use in the treatment of diarrheal diseases.

The extent of potency of Ca blockade by the various agents is likely affected in part by the dependence of a particular tissue or cellular process on the supply of extracellular Ca. Other variables including the number and type of Ca channels, and the membrane potential play a role in determining the susceptibility of a tissue to a CCB (Spedding, 1988).

The widely recognized pharmacologic effects of CCBs include coronary, peripheral and cerebral vasodilation, a negative inotropic effect, and inhibition of excitation of sinoatrial

and atrioventricular nodes. Based on these effects, CCBs have become important clinical agents in the treatment of angina pectoris, hypertension, posthemorrhagic cerebral vasospasm and supraventricular tachycardia (Vaghy, 1987). Other potential uses include inhibition of platelet aggregation, relief of migraine and asthma and protection of ischemic myocardium.

The effect of CCBs in reducing Ca movement from the extracellular space across the membrane has important implications for Ca as a second messenger and therefore has the potential to affect several cellular functions. As indicated above, the influx of extracellular Ca is heightened during many of the cellular processes activated by the Ca messenger system.

New derivatives of these agents are continually being developed and CCBs are being widely studied. Their effects on Ca influx combined with the potential varied tissue specificity could have important consequences. It is likely that the list of clinical benefits and experimental significance of this important class of agents will continue to grow with ongoing research.

1.10) CALCIUM AND INTESTINAL REGULATION

1.10.1) IONIC TRANSPORT

At least two ionic transport mechanisms appear to be affected by alterations in the intracellular level of Ca in small intestinal tissues, particularly the ileum. These include linked Na and Cl absorption and Cl secretion (Donowitz and Welsh, 1986). A brief review of these ion transport mechanisms and a more lengthy discussion of their regulation by Ca is included in the following section. The emphasis is upon regulatory mechanisms as some of these processes may have potential significance in terms of nutrient uptake and Ca. Since a great deal of the experimental evidence providing support for a role of Ca has been obtained using the Ussing chamber technique, it is also described in a separate section.

1.10.2) SODIUM AND CHLORIDE TRANSPORT IN THE SMALL INTESTINE

Na and Cl transport is influenced by CCBs as discussed in section 1.10.4. Thus, a review of these transport processes is relevant. Na may be absorbed in the jejunum by several processes including:

A) Na-substrate cotransport. This will be discussed more fully in the context of hexose absorption.

B) Diffusion pathways.

C) Co-transport with PO4 and SO4.

D) Na/H exchange

A summary of potential transport mechanisms in the jejunum is depicted in Figure 1-2.

The entry of Na is favoured by the low intracellular Na content of the absorptive epithelial cells which in turn is maintained by the activity of the Na,K-ATPase in the BLM. In addition, a high intracellular K concentration and a negative cell voltage are maintained, contributing to the large electrochemical gradient for Na entry.

Na/H exchange has been demonstrated using manipulation of the proton gradient in BBM vesicles isolated from rabbit jejunum (Gunther and Wright, 1983). This electrically neutral process that operates in the absence of an electrical gradient also appears to be present in rat and human jejunum (Donowitz and Welsh, 1987). The Na/H exchange mechanism likely accounts for a small percentage of the total Na flux in jejunum; an estimated 7% of total Na flux in the absence of food. It has been proposed that the most important pathways for Na flux across the jejunal BBM membrane are diffusion and organic

substrate/Na co-transport systems (Gunther and Wright, 1983). However, the precise mode of diffusive Na entry has not been completely defined.

It has also been proposed that the Na/H exchange mechanism may be a major mechanism for jejunal bicarbonate (HCO_3) absorption (Powell 1985; Gunther and Wright, 1983). The secreted H can combine with HCO_3 in the lumen to form carbonic acid (H_2CO_3) which further dissociates to carbon dioxide (CO_2) and H_2O; the former of which diffuses across the apical membrane and reforms HCO_3 via carbonic anhydrase. Thus the overall process results in $NaHCO_3$ absorption.

Active Cl absorption does not appear to occur in mammalian jejunum (Gunther and Wright, 1983). However in ileum, Na and Cl absorption is a linked or coupled process. Nellans et al (1973) and others have observed a codependency of these two ions for transport across BBM. Subsequent studies with vesicles have suggested that the coupling of Na and Cl in the ileum is indirect. The linkage is likely due to the operation of two transport exchangers operating in parallel in the apical membrane; a Na/H exchanger and a Cl/HCO_3 exchanger. The mechanisms of linking between the two exchangers have not been defined. In ileal brush border vesicles, alterations in intravesicular pH have been suggested as a potential mode of linkage, however, whether this occurs physiologically has not been determined.

Thus, Cl absorption in the ileum is likely accomplished by this Cl/HCO_3 exchange mechanism in addition to passive transport down the electrical gradient through the paracellular shunt pathway. The rate limiting step for the absorption of Na and Cl is likely the apical entry step at the BBM. The majority of studies have concluded that the Na,K-ATP pump is the primary mechanism of Na exit across the BLM (Powell, 1985). The electrogenic pump has a stoichiometry of 3 Na:2 K.

Cl entry across the apical membrane results in elevated intracellular concentrations however the Cl permeability of the BLM may be limited. Carrier-mediated exit across this membrane involving Cl/HCO_3 exchange likely enhances the rate of transepithelial Cl movement.

It is likely that absorption occurs via absorptive cells present on the villus of the small intestine (and on the surface of the colon). From microdensitometry studies it appears that the upper third of the villus is the major site for amino acid transport (Cheeseman, 1986). Phlorizin-binding studies provide similar evidence for glucose uptake (Chang et al, 1987). In contrast, secretory cells appear to be present mainly in the crypts (Donowitz and Welsh, 1987). Thus, crypt cells are likely involved in Cl secretion (i.e. movement from blood to the lumen) in the small intestine. In several non-mammalian cell types it is proposed that Cl crosses the BLM via a co-transporter with Na and K. The carrier is electroneutral and the stoichiometric ratio is likely 1 Na:1 K:2 Cl (O'Grady et al, 1987). The Na pump provides the driving force for the entry of Cl by co-transport with Na. The movement of Cl across the luminal membrane is thought to occur through channels that are opened by cyclic adenosine monophosphate (cAMP) or possibly Ca (Field et al, 1989). Thus, net Cl secretion is accomplished by the Na-coupled electroneutral transport mechanism in the BLM and the Cl channels in the BBM. In general, the conductive permeability of the luminal membrane to Cl is the rate limiting determinant of net secretion. The secretion of Cl via the transcellular route is accompanied by the movement of Na in the same direction via the paracellular pathway (Field et al, 1989).

1.10.3) USSING CHAMBER TECHNIQUE

The Ussing chamber technique has been widely used to measure transepithelial electrical parameters and ion fluxes in intestinal tissue and other epithelia (Ussing and

Zerhan, 1951). Ussing chamber studies of jejunal tissue were also undertaken in the present study to determine the effects of CCBs on basal and stimulated ionic transport. For intestinal studies, intact sheets of tissue are stripped of external muscle layers to permit adequate oxygenation and to reduce the effect of the muscle layer contribution to tissue resistance (White, 1977; Frizzel et al, 1974). The intact segments are then mounted vertically as a flat sheet between two sides of a divided chamber. Thus, mucosal and serosal surfaces are exposed through an aperture of known diameter to circulating and oxygenated experimental solutions. This set up allows the addition of various test agents (drugs, nutrients etc.) to either the mucosal or serosal side of the chamber.

Salt bridges are connected to external electrodes. Two of these salt bridges (connected through reversible electrodes to a voltage recording apparatus) are positioned as close as possible to either side of the tissue to monitor transepithelial potential. Although the distance between the sensing bridge and the tissue is minimal the fluid resistance contributed by this space may be significant and therefore is compensated for manually or automatically in most current cases.

Two other bridges are connected to a current source thus allowing controlled amounts of current to be passed across the tissue. Thus, the technique allows measurements of the spontaneous potential difference (PD) across a tissue (open circuit). Additionally, the amount of external current required to bring this spontaneous PD to zero (closed circuit) may also be determined. In the latter state (PD of zero) and when mucosal and serosal solutions are identical, all known physicochemical driving forces across the membrane are eliminated. Thus, the external current required to maintain a zero PD represents the algebraic sum of the net fluxes of ions that are actively transported in both directions across the tissue. This measurement has been termed "short circuit current" or Isc

and is widely used in the following literature. Specific directional fluxes and the relative contributions of individual ions to the total Isc may be determined by using radiolabelled ions.

The Ussing chamber is a useful technique however it is limited in that it does not provide information regarding specific involvement of various components of the intact tissue. The recent literature using vesicles of select membranes has helped to provide more specific information regarding potential mechanisms and cellular components involved.

1.10.4) CALCIUM EFFECTS ON ION TRANSPORT

A regulatory role for cytosolic Ca has been suggested by numerous studies using both intact tissues and later, isolated membrane preparations. In general, experimental manoeuvres expected to increase the concentration of Ca (mainly via serosal entry) in the cell cytosol are associated with a reduction in Na and Cl mucosal to serosal transport (absorption) and/or an increase in electrogenic Cl movement from serosa to mucosa (secretion).

Rabbit ileum has been extensively investigated. In 1977, Bolton and Field added Ca ionophore A23187 (to enhance Ca entry) to solutions bathing rabbit ileal segments in Ussing chamber experiments. They reported a decrease in net Na and Cl transport and an increase in Isc. Similar findings were observed by adding excess Ca (10mM) to the external bathing solutions.

Subsequent work with BBM vesicles agreed with the results of the intact tissue experiments suggesting that at least part of the regulatory effect occurs in the apical membrane of the ileum (Fan et al, 1983). Furthermore, this work demonstrated that the Ca-mediated inhibition of Na uptake was Cl-dependent and vice versa. Glucose and amino acid-stimulated uptake of Na does not appear to be affected by altered levels of Ca in the

above studies. Specifically, in calcium deprived bathing solutions, the 10 mM (mucosal) glucose-dependent increase in Isc was not significantly different from controls (Donowitz and Asarkof, 1982). However, one author has reported that increased intravesicular Ca decreased Na dependent glucose and amino acid uptake (Fondacaro and Madden, 1984). Whether this was a specific effect or due to alterations in Na gradients or nonspecific interactions has not been clarified.

Similarly, elevated levels of Ca also affected various aspects of NaCl transport and Isc in other tissues including rabbit colon, rat colon and flounder intestine (Donowitz, 1983). In some of these tissues, the effect was primarily a stimulation of Cl secretion.

Conversely, lowering external Ca in the bathing solutions surrounding rabbit ileal tissue has been shown to increase mucosal to serosal movement of Na and Cl. Studies lowering external Ca to 50 μm have been associated with increases in Na and Cl absorption; in addition, Isc decreases, presumably attributed to decreases in residual ion flux which is likely reduced bicarbonate secretion in the ileum (Donowitz and Asarkof, 1982; Donowitz et al, 1984). This phenomenon (i.e. lowered to Ca stimulation of ion transport in small intestine) has only been confirmed in rabbit ileum. Again, interdependence of Na and Cl transport was observed and glucose-stimulated Na was not affected.

Donowitz and his group have reported all of the three major classes of CCBs (phenylalkylamines, dihydropyridines, and benzodiazepines) have been shown to stimulate linked Na and Cl absorption in rabbit ileum (1982, 1985, 1989). In general these agents appear to exert this effect mainly when applied to the serosal side of the tissue (see section 1.10.5).

The potential physiological significance of Ca regulation in electrolyte transport is supported by the observation that several neurohumoral substances normally present in the

intestinal tissue appear to regulate electrolyte transport by increasing cytosolic Ca. For example, carbachol, serotonin, substance P, and neurotensin have all been shown to increase the rate of [45Ca] entry across ileal BLM and increase total ileal Ca content (Donowitz, 1983). All of these substances reduce Na and Cl absorption and/or induce Cl secretion. Furthermore, these effects of stimulated transport are inhibited by decreasing serosal Ca or by serosal verapamil. Therefore, Ca likely acts as the intracellular mediator for the action of these neurohumoral substances.

1.10.5) MECHANISMS OF CALCIUM REGULATION

The above findings suggest that plasma membrane entry of Ca is likely an important event in regulating both basal and stimulated electrolyte transport. Based on studies using CCBs, it appears that serosal Ca entry is of particular significance. The addition of verapamil to mucosal and serosal surfaces of rabbit ileum does not produce any effects different from serosal exposure alone (Donowitz and Asarkof, 1982). The presence of verapamil significantly reduces [45Ca] entry across the BLM but not mucosal Ca entry (Donowitz, 1983). Furthermore, reducing Ca concentrations in mucosally exposed solutions does not affect basal Na and Cl transport. The different effects may due to fewer Ca channels in the BBM or binding or vesiculation of absorbed mucosal Ca. Whether these observations are unique to rabbit ileum and why serosal Ca entry appears to be more regulatory is unclear. Luminal Ca in rat small intestine may in fact play a role in regulating transport of Na (Markowitz et al, 1985). In vivo jejunal perfusion of 0.4 mM V dose-dependently stimulated luminal mucosal to serosal Na water flux (Markowitz et al, 1985). At higher concentrations, however (1.6 mM) the effect appeared to be reversed (i.e. secretion). This study was reported in abstract form only.

The observation that CCBs have the effect of increasing basal Na and Cl transport suggests that there are Ca channels in the BLM and that these may be partially open in the resting state. It is hypothesized that the neurohumoral substances affecting Ca entry (carbachol, serotonin, substance P and neurotensin) likely function by binding to the plasma membrane to trigger messages that alter cellular Ca handling and the state of BLM Ca channels. This would be similar to the mechanism of receptor operated Ca channels (ROCC) stimulation described previously. As suggested earlier, it is unknown if VDCC differ from ROCC or if hormonal binding mediates alterations in the properties of ROCC.

A more detailed demonstration and characterization of BLM Ca channels was reported by Homaidan et al (1989). This group used CCBs from the three major groups (including phenylalkylamines, dihydropyridines and the benzothiazepine, diltiazem) to characterize Ca channels in rabbit ileum and enriched BLM preparations of this tissue. As previously demonstrated using the Ussing chamber technique, serosal addition of all classes of drugs dose-dependently decreased Isc, likely due to increased Na and Cl absorption, and reduced residual ion flux. However, the phenylalkylamines had a further inhibitory effect on Isc even in the presence of maximally inhibiting concentrations of dihydropyridines. This might be suggestive of a distinct channel or site with different binding kinetics for the latter drug. Maximal and half-maximal inhibitory concentrations varied among classes, with verapamil (phenylalkylamine) being the most effective inhibitor of basal Isc.

Although the effects on Isc suggest two potential binding sites, radioligand binding was characteristic of a single, saturable, high affinity binding site for phenylalkylamine structure using the derivative [^{3}H(l)-desmethoxyverapamil]. The authors (Homaiden et al, 1989) suggest that the binding affinities may differ between the purified membranes and the

intact cell. Thus, further evidence is required to characterize a potential second channel or binding site for this group of CCB.

Dihydropyridine binding sites (using [^{3}H-nitrendipine]) also exhibited saturable, high affinity single site binding characteristics. In addition, these radioligand studies also indicated that there are three times as many binding sites for phenylalkylamine structures than the dihydropyridines. Displacement studies showed that the three classes of drugs do not classically compete for binding sites in the ileum. Rather, similarly to other tissues there appears to be allosteric interactions at the binding site(s) by the various agents.

This study provides good evidence for CCB binding sites and apparent VDCC in small intestinal epithelial cells. The authors conclude that two distinct channels exist; an L-type channel that may bind dihydropyridines, phenylalkylamines and benzothiazepines at three different sites and a second channel that exclusively binds the phenylalkylamines. However, further study is warranted.

In addition to plasma membrane entry, there is indirect evidence that intracellular Ca stores may play a role in the regulation of some aspects of electrolyte transport (Donowitz, 1983). Drugs which are shown to confine Ca within intracellular stores of the cell (e.g. dantrolene) have been tested in rabbit ileum. Serosal exposure to dantrolene results in stimulated Na and Cl absorption but again does not affect glucose-dependent uptake of Na. In terms of stimulated transport, these drugs inhibit the effects of carbachol but not serotonin raising the possibility of different modes of Ca mobilization by these two substances (Donowitz and Welsh, 1986).

The precise molecular mechanisms involved in the regulation of electrolyte transport by Ca are not completely understood; recently mechanisms are being proposed. Although

serosal Ca entry is important, most evidence suggests that the apical membrane is likely a key factor in the regulatory mechanisms.

Many Ca-regulated cellular functions in other tissues are CAM-dependent and this also appears to be the case for electrolyte transport in rabbit ileum. CAM is present in the brush border and the soluble fraction in epithelial cells. Using BBM vesicles, Powell and Fan (1984) demonstrated that preincubating vesicles with 20 μm CAM shifted the dose-response curve for Ca-mediated inhibition of basal Na and Cl uptake (i.e. a significantly lower concentration was required for half maximal inhibition). Antagonists to the Ca-CAM complex have also supported the involvement of CAM. Specifically, the naphthalenesulfonamide W13 has been used for this purpose, as well as a hydrophobic control W12 which has an almost identical structure, but not Ca-CAM inhibiting activity (Donowitz et al, 1989). The presence of 45 μm W13 decreased ileal Isc and stimulated mucosal to serosal fluxes of Na and Cl. The net effect was significantly increased absorption of Cl compared to the lack of effect by the hydrophobic control W12, (a naphthalenesulfonamide that differs from W13 by a single chlorine atom). Glucose dependent Na absorption was not affected, nor were the stimulated alterations in electrolyte transport caused by Ca ionophores, serotonin, or manipulations that increase cyclic AMP (Donowitz and Welsh, 1986; Donowitz et al, 1989).

In most nonintestinal tissues increased cytosolic Ca leads to several Ca-CAM directed phosphorylation reactions and in this way regulates several aspects of cell function. Protein phosphorylation has been suggested to modulate the function of other transport proteins including ion pumps and voltage dependent channels. The presence of endogenous Ca and CAM dependent protein kinases has been suggested in the ileum. Experiments with purified apical membranes show that varying concentrations of Ca or Ca plus CAM dose-dependently

increase the phosphorylation of five peptides by 50-110% (Donowitz et al, 1984). These peptides have been identified by one dimensional SDS-PAGE and autoradiography. The presence of Ca-CAM versus Ca alone results in larger increases in phosphorylation and less variability in the results. Furthermore, inhibitors of the Ca-CAM complex inhibit the enhanced phosphorylation.

Recent investigations have focused on the possibility of phosphorylation and have attempted to determine more specifically which aspects of Na and Cl transport might be substrates for phosphorylation reactions. Rood et al (1988) have studied the regulation of the Na/H antiporter in rabbit ileal BBM vesicles. These authors used a previously validated technique to incorporate macromolecules and to alter the proton gradient across the vesicle to differentiate between Na/H exchange and other Na transport mechanisms. Their study showed that the combination of Ca, CAM and ATP inhibited Na uptake when there was an acid inside pH gradient (presumably the Na/H mechanism) while in the absence of a proton gradient Na uptake was not affected by this combination. Replacement of ATP with a nonhydrolyzable analogue was also without effect thus implicating phosphorylation via Ca-CAM as an effect on the Na/H exchange process in rabbit ileal membrane.

In a subsequent study, Emmer et al (1989) used the same technique in rabbit ileum to determine the kinetics and specificity of the Ca-CAM,ATP inhibition of Na/H exchange. In these studies diffusive Na uptake, and Na-dependent glucose uptake were not affected. They reported that the inhibitory effects of Ca-CAM and ATP on Na/H exchange occur under conditions which are similar to Ca-dependent phosphorylation of membrane proteins in the same vesicles. For example, Ca-CAM phosphorylation of five apical membrane occurs with a 50% effect at a mean cytosolic free Ca concentration of 105 nM and maximal effects at 300-800 nM (with decreased phosphorylation above this maximum). Similarly, 50% of

maximum inhibition of the Na/H exchanger by Ca-CAM and ATP occurs with cytosolic Ca concentrations of 120 nM and maximal inhibition at 300-850 nM. Furthermore, these reported Km's are close to the estimated cytosolic Ca concentration (determined using a Ca-sensitive dye Fura-2) of 130-140 nM in resting ileal cells suggesting that small alterations in cellular Ca concentration could regulate Na/H exchange in the basal physiological state.

However, the imposed proton gradients in these vesicle studies may not represent the in vivo conditions. Furthermore, isolated membrane does not necessarily represent the situation in whole tissue. In one study, intact segments of ileum were exposed to conditions associated with increased Ca entry (Ca ionophore A23187). Subsequently, phosphorylation of peptides was determined on whole cell homogenates and BBM from these tissues. No significant changes in protein phosphorylation were detected (Powell and Fan, 1984).

However, the majority of current research does support a role for phosphorylation in the regulation of electrolyte transport, at least in rabbit ileal BBM. Ca-CAM dependent protein kinases have been identified in chick small intestinal brush border vesicles (Caraboni et al, 1987). Improved purification of cell fractions containing the appropriate substrates and refined peptide techniques are likely important in detecting the small changes in phosphorylation occurring in intact tissues.

Studies have also been done to determine if the mechanisms of regulation under basal conditions differ from regulation of stimulated transport processes. Recent research suggests that basal absorptive processes may be under the continuous inhibitory control of Ca-CAM while transport responses to elevated intracellular levels of Ca are likely mediated through an increase in protein kinase C.

Donowitz et al (1989) used an assay of cytosolic protein kinase C (PKC) to determine effective conditions under which a PKC inhibiting drug, H-7 (isoquinolenesulfonamide),

would decrease ileal villus PKC with no affect on Ca-CAM dependent kinases. Extrapolating these conditions to intact tissue in Ussing chambers, these authors found that 60 μm H-7 did not alter Isc or Na and Cl fluxes under basal conditions or after addition of 10 mM glucose to the mucosal chamber. However H-7 did affect the electrolyte transport that was stimulated by Ca-elevating agents including carbachol, serotonin and Ca ionophore A23187. Specifically, H-7 partially inhibited the usual increase in Isc and decreased Na and Cl fluxes normally caused by these agents.

Conversely, 45 μm W13, the inhibitor of Ca-CAM, as previously shown, caused significant decreases in Isc and increased mucosal to serosal fluxes of Na and Cl under basal conditions. However, under carbachol and Ca ionophore-stimulated conditions, this agent did not affect Cl transport. Combinations of the two inhibitors did not demonstrate additivity or synergism in any of their effects.

Thus, these authors concluded that the regulatory effects of raised cytosolic Ca concentrations are mediated by PKC while basal electrolyte transport is mediated by Ca-CAM, possibly via protein kinases dependent on this complex. The proposal of sequential regulation in this case is not unlike the two branch model of Ca regulation (ie CAM branch and PKC branch) proposed by Rasmussen (1984, 1986) (see "Calcium Messenger System").

In conclusion, the mechanism of how changes in intracellular Ca concentrations might alter apical membrane ion permeability are not completely understood. But clearly, continuing research is providing significant clues to regulatory mechanisms involving this ion. It must be recognized however, that most studies have investigated the regulation of ion transport in rabbit ileal tissue. Whether these findings are generally applicable to regulation in the jejunum and other tissue of the small intestine and in other species is unknown.

Secondly, many of these studies have focused on the short term effects (usually a few hours or less) of manipulating Ca in the tissue environment as well as intracellular Ca levels. The effects of chronic alterations, for example the long term administration of CCBs on regulation of transport in the small intestine have not been reported. It is unknown how CCBs might influence intestinal membrane composition, morphology and nutrient transport function (see section on "Intestinal Adaptation"). Although Na-dependent glucose uptake is not affected by altering Ca levels in these short term studies, direct in vitro measurement of the substrate uptake itself has not been determined. Furthermore, direct effects of the CCBs on the intestinal uptake of lipids and sterols have not been determined.

In addition to stimulating ileal electrolyte absorption, CCBs have a similar affect on water absorption in the ileum and colon (Donowitz et al, 1985). The dose of verapamil that causes maximal stimulation of water absorption is similar to a standard cardiac therapeutic dose of 240 mg for a 70 kg adult. Thus, the effects of CCBs on water and electrolyte absorption suggest a potential clinical use for this drug in the treatment of diarrheal diseases. Cardiac and non-cardiac adverse effects of CCBs have been reported in hypertensive patients treated with chronic administration of verapamil. The most commonly reported side-effect is constipation; others less common effects include headache, dizziness, fatigue, flushing and nausea (McTavish and Sorkin, 1989). Cardiac adverse effects reported include bradycardia, palpitations and orthostatic hypotension. Similar effects have been reported for other classes of CCBs.

1.10.6) THE PARACELLULAR PATHWAY AND CALCIUM

A conductive pathway for ions and solutes in parallel with the transcellular pathway has been demonstrated in the small intestine and other epithelial tissues. Electron microscopy and freeze fracture studies have shown that there are apparent fusion sites

between adjacent epithelial cells characterized by linear interconnecting strands or grooves at the fusion points. Ions and water may move passively through these junctional spaces between cells which are collectively referred to as "tight junctions"; the entire conductive pathway has been designated the extracellular or paracellular shunt pathway.

The ionic conductance of this paracellular pathway accounts for approximately 90% of the total tissue conductance in small intestine (Armstrong, 1987). Thus, alterations in the properties of this highly conductive pathway may have important effects on the properties and potential difference across the entire tissue as well as potentially affecting transcellular transfer of solutes.

The composition of the interconnected "strands" of the tight junctions is unknown (Madara, 1989). Both lipid and more likely, protein components have been postulated. These structures have been proposed to represent a resistive barrier within the tight junction and thus potentially determine the functional characteristics of the junctions. Although subject to criticism, it has been proposed that "leaky epithelial systems" are characterized by fewer strands (and thus lower resistance and higher conductance) while "tight epithelia" have multistranded tight junctions (Madara and Marcial, 1984). However, in the small intestine, tight junction structure appears to be variable between crypt and villus cells and within each cell type. Thus, this structure-function relationship may be somewhat simplified for heterogenous tissues.

Tight junctions exhibit rapid structural and functional changes in response to changes in extracellular and intracellular environments. Under normal circumstances, the paracellular pathway is cation selective and demonstrates a characteristic selectivity pattern for several monovalent ions. In jejunal villus absorptive cells, exposure to mucosal osmotic loads resulted in increased transepithelial resistance, reduced cation selectivity and a subpopulation

of absorptive cells acquired additional tight junction strands (Madara and Marcial, 1984). Exposure to various buffers and cyclic AMP also affect structure and function; in the latter case the effects were reversible.

In several epithelia, including small intestine, depletion of extracellular Ca by Ca chelators has altered tight junction structure and/or function (Donowitz and Madara, 1982; Madara and Marcial, 1984). These effects appear to be specific to Ca and may be reversed upon addition of this ion to the solution bathing the tissue.

A variety of techniques have demonstrated mixing of apical and basolateral domains following depletion of extracellular Ca possibly due to disruption in junctional strands (Madara and Marcial, 1984). Under normal circumstances these structures likely provide barriers to prevent lateral diffusion of proteins between apical and BLM. Lowered extracellular Ca in the external environment also produces decreased transepithelial resistance and in some tissues alterations in charge selectivity. In the studies of Na and Cl regulation reported above, depleting extracellular Ca irreversibly increased the conductance of the tissues studied. However histologic and functional parameters appeared to be normal (Donowitz and Asarkof, 1982). It is uncertain what effects CCBs have on the tight junctions or if intestinal exposure to these agents would reduce Ca levels enough to alter transepithelial resistance.

The mechanisms responsible for the Ca-mediated and other reported effects on the paracellular pathway in the small intestine are unclear. One proposal suggests that the cytoskeleton of epithelial cells may interact with the tight junctions and that altered properties and functions of the junctional complexes are mediated via effects on the cytoskeleton (Madara, 1989). However, more work is needed to clearly define the nature of the relationship between tight junction structure/function and Ca concentration. The

effects of chronic alterations in cellular Ca such as with the long term administration of CCBs is unreported.

1.11) ATHEROSCLEROSIS

1.11.1) GENERAL

Early definitions of atherosclerosis generally refer to a degenerative process involving progressive hardening of the blood vessel wall. Today it is recognized that atherosclerosis is the final common result of several complex interactions between cells of the arterial wall and a number of blood components. Alterations in adhesion, migration, proliferation and transformation of several cell types including platelets, monocyte-macrophages, endothelial cells and smooth muscle cells are involved (Steinberg, 1987).

The interaction of the pathogenic pathways is complex and several theories have been developed to explain the sequence and manner in which atherosclerosis develops and progresses at the cellular level. Although there is no universal agreement there are two theories of atherosclerotic development and particularly initiative events that are widely quoted. The "response-to-injury theory" originally put forward by Ross and Glomset (1976) and recently modified by Ross (1986) implicates endothelial physical and functional injury as a crucial event in the development of atherosclerosis. Several events may alter the state of the endothelium including elevated LDL levels and hypertension. The "lipid theory" of Brown and Goldstein (1984) proposes that excess deposition of lipid in the vessel wall initiates the atheroma. The apparent overlap and detail of these two theories becomes evident in the following discussion.

Two major "forms" of atherosclerotic lesions have been described including the fatty streak and the fibrous plaque. Based on recent morphological studies involving diet-induced

hypercholesterolemic nonhuman primates (Faggiotto et al, 1984; Faggiotto and Ross, 1984) it has been observed that there is an early sequence of cellular events leading to the development of a fatty streak similar to that seen in humans. These include in summary: 1) adherence of circulating monocytes to the endothelium; 2) transendothelial migration of these monocytes; and 3) accumulation of monocyte-derived lipid filled macrophages (i.e. foam cells) in the subendothelium. Thus, the fatty streak is characterized by the accumulation of these foam cells and a variable portion of smooth muscle cells (SMC), as well as T-lymphocytes, and an extracellular matrix of lipid, collagen, elastin and proteoglycans.

This fatty streak is non-obstructive and probably forms the precursor for the more advanced fibrous lesion. With continued cholesterol feeding in nonhuman primates (5 months) there is continued subendothelial monocyte migration and foam cell accumulation as well as the gradual movement and proliferation of SMC from media to intima.

The endothelial cells may retract (i.e. denudation) exposing the underlying connective tissue and macrophages. According to the "injury hypothesis" the interaction of the early lesion with platelets that release platelet derived growth factor (PDGF) is the important stimulating event to initiate SMC proliferation and migration. However, macrophages, endothelial cells and SMC themselves also produce PDGF as well as other growth factors and chemoattractants. Thus, as proposed in a modified version of the initial theory, denudation does not need to occur as growth factors from the above cells likely mediate SMC changes in areas where the endothelium remains intact (Ross, 1986).

Thus, the progression of atherosclerotic lesions involves the continued interaction of the SMC (the major cell type in the fibrous plaque) and a number of factors produced by the arterial wall. More study is required to determine the relative importance of the various cells and growth factors in the in vivo situation.

The composition of the lesion may vary but is generally characterized by a necrotic core of cellular debris, lipid, cholesterol, Ca deposits, with overlying SMC, macrophages and T-lymphocytes, capped with a fibrous layer of SMC, leukocytes and connective tissue (Ross, 1986).

1.11.2) HYPERLIPIDEMIA AND ATHEROSCLEROSIS

The relationship between hyperlipidemia and atherosclerosis has been well established by a wealth of experimental, clinical, genetic, epidemiologic and intervention data. The cholesterol that accumulates in the atherosclerotic lesions originates primarily from the lipoproteins in the circulation. While it is generally agreed that the cholesterol in the LDL and β-VLDL blood fractions are atherogenic, the exact mechanisms of their role are not yet conclusive (Steinberg, 1987).

Chronic exposure to elevated circulating LDL may have a direct injurious affect upon the endothelium. Jackson and Gotto (1976) have suggested that alterations in the cholesterol:PL ratio of plasma membranes in epithelial cells may increase membrane viscosity thus altering the nature of the cell, resulting in denudation and possibly promoting monocyte adhesion. Others have reported that excessive cholesterol feeding alters the normal endothelial property of repulsing thrombocytes and monocytes (Ross, 1986). The LDL may also play a role by interacting with other systems that favour lesion formation such as increasing aggregation of platelets. In addition, excessive LDL may promote more rapid release of growth factors from arterial cells (Yatsu and Fisher, 1989).

In accordance with the "lipid theory", elevations in cholesterol-carrying lipoproteins may result in increased uptake of cholesterol across the arterial wall. As outlined in the "lipoprotein metabolism" section, the LDL receptor is involved in the highly regulated entry

of extracellular cholesterol to both hepatic and extrahepatic cells as described by Brown and Goldstein (1986).

In vitro incubation of monocyte-macrophages with high concentrations of LDL does not promote excessive deposition of cholesterol in these cells. This led Brown and Goldstein (1984) to postulate that alternate receptor mechanisms that bind modified forms of LDL are operative in the formation of monocyte-derived foam cells. Their studies demonstrated that chemical acetylation of LDL resulted in enhanced uptake into murine macrophages by a saturable, specific, receptor mechanism. Unlike the native LDL receptor, the activity of this "scavenger cell receptor" is not downregulated by the intracellular content and thus continues to accumulate the modified LDL. These CE-filled macrophages are reportedly similar to the in vivo foam cells in many respects. Other chemically modified forms of in vitro LDL have demonstrated similar activity through alternate pathways (Steinberg et al, 1989; Haberland and Fogelman, 1987).

A modified form of LDL in vivo has not been directly demonstrated, however convincing arguments for oxidized LDL as a potential candidate have been presented (Steinberg et al, 1989). It has been proposed that peroxidation of the fatty acids in LDL lipids may initiate a series of events that directly modify the LDL apo-B (possibly critical lysine residues) to produce a form recognized by the scavenger cell receptor (Haberland and Fogelman, 1987). Several properties of the oxidized LDL (chemoattractant for monocytes, cytotoxic effects and inhibitor of macrophage motility) suggest a potential role in the atherogenic process. Furthermore, it has been suggested that Probucol (a lipid lowering agent and potent anti-oxidant) may inhibit atherosclerosis independently of its lipid lowering effects (Carew et al, 1987).

Although several of these assertions may be challenged and further study is needed, the potential role of lipoprotein alteration as an event associated with pathogenesis of atherosclerosis is an intriguing possibility. Furthermore, the presence of alternate or nonreceptor-mediated uptake mechanisms for cholesterol may be quantitatively significant in some tissues (Meddings et al, 1987; Dietschy, 1984). These sites may also represent a potential target of regulation by Ca or CCBs.

1.11.3) CALCIUM AND ATHEROSCLEROSIS

It is well established that the Ca content is increased in atherosclerotic arteries. Classically, it has been suggested that enhanced calcification might be a secondary event in the atherosclerotic process. Recent evidence however, has implicated Ca as a causal component (Strickberger et al, 1988). Specifically, it has been speculated that intracellular Ca may act as a pathogenic second messenger in the atherosclerotic process (Phair, 1988). This suggestion has been stimulated by the observation that enhanced Ca entry may occur as an early event in the initiation of the atherosclerotic lesion. The Ca accumulates intra and extracellularly. However, the consequence of the intracellular Ca accumulation and how this behaves as a second messenger is unknown.

These speculations and observations have led to extensive experimental work with CCBs in an attempt to manipulate the cellular influx of Ca and determine the resulting impact on the atherosclerotic development.

As described further in "Chapter 2" (Introduction), the administration of CCBs from each of the three major classes including phenylalkylamines, dihydropyridines and benzodiazepines has been shown to be antiatherogentic in several animal models. It is currently thought that these agents do attenuate the development of atherosclerotic lesions. However, there have also been negative reports in the literature, which have created

confusion and a lack of consensus regarding the effectiveness of these agents and the exact role of Ca in atherosclerosis. Furthermore, there is little consistency in the literature in terms of animal models, drug doses and administration routes; thus, cross-comparison of the reported results is difficult.

Nonetheless, several mechanisms for the putative antiatherosclerotic effects of CCBs have been proposed. A list of the potential sites where Ca might be involved in the pathogenesis of atherosclerosis and that may be altered by CCBs, is provided in Table 1-1.

AIMS OF RESEARCH

In view of the previously described background, a research project was designed with the following specific aims:

A) To test the hypothesis that there are multiple Ca channels in the jejunum.

B) To determine if Ca channels play a role in active or passive uptake of hexoses and lipids in the jejunum.

TABLE 1-1: Partial list of calcium-regulated processes at the arterial wall level that may be altered by calcium antagonists.

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(Weinstein and Heider, 1987)

FIGURE 1-1: The calcium messenger system initial and sustained branch.

The shaded area in each panel represents the plasma membrane. During the initial phase (left), the main flow of information is through the calmodulin (CaM) branch of the system. Hormone-receptor interaction leads to the hydrolysis of phosphatidylinositol-4,5-bisphosphate (PIP_2), giving rise to diacylglycerol (DG) and inositol 1,4,5-trisphosphate (IP_3). The water-soluble IP_3 stimulates the release of Ca^{++} from the endoplasmic reticulum, causing a transient rise in the concentration of Ca^{++} ($Ca^{2+}c$). This rise in the Ca^{++} concentration is responsible for activating CaM-dependent protein kinases. These enzymes catalyze the phosphorylation of a subset of cellular proteins (Pr_s·P) that are responsible for the initial phase of the cellular response. The increase in the DG content of the plasma membrane, along with the transient rise in the Ca^{++} concentration, brings about the shift of protein kinase C from its Ca^{++}-insensitive form [CK] to its Ca^{++}-sensitive form (CK) Hormone-receptor interaction also leads to stimulation of the rate of Ca^{++} influx ↓ and, secondarily, to the CaM-dependent activation of the plasma membrane Ca^{++} pump ↑. This increase in Ca^{++} cycling leads to an increase in the amount of Ca^{++} within a submembrane cellular domain [Ca^{++}] which regulates the activity of the Ca^{++}-sensitive form of protein kinase C. During the sustained phase of the response (right), the Ca^{++} concentration falls back to its basal value, and the activity of CaM-dependent protein kinases decreases so that this subset of cellular proteins (Pr_s·P) is no longer phosphorylated. However, the increases in plasma membrane Ca^{++} cycling and in DG are sustained and are responsible for the sustained activation of C-kinase. C-kinase, in turn, catalyzes the phosphorylation of a second subset of cellular proteins (Pr_t·P) that are responsible for controlling the sustained phase of cellular response.

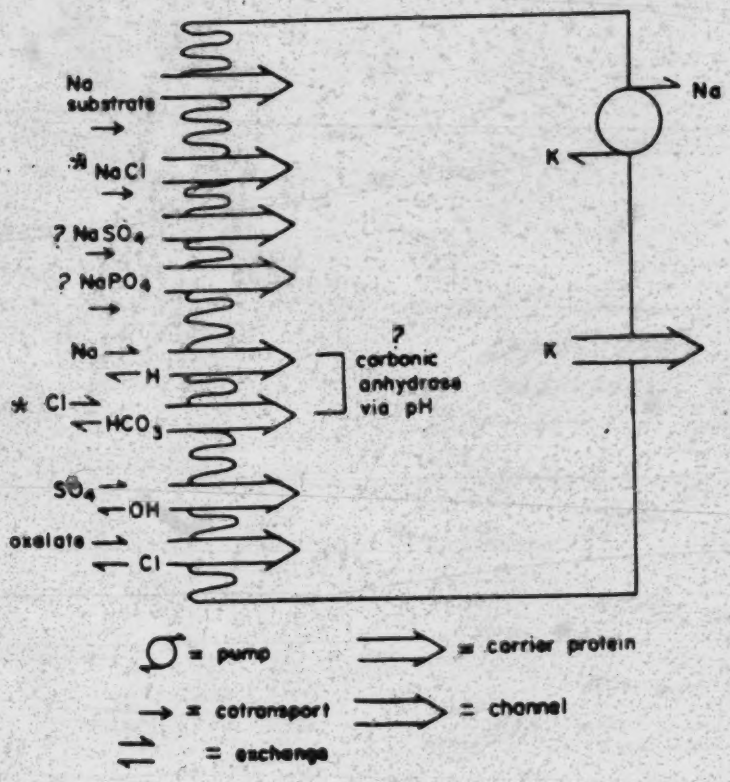
Note: Model based on Angiotensin II Action on Adrenal Glomerulosa Cells (Rasmussen 1986a).

FIGURE 1-1

DIAGRAM OF CALCIUM MESSENGER INITIAL AND SUSTAINED BRANCH HAS BEEN OMITTED FROM THE THESIS DUE TO COPYRIGHT PROTECTION

(Rasmussen 1986a)

FIGURE 1-2: Sodium transport processes in the jejunum.



(Adapted from Donowitz and Welsh, 1987)

*Experimental evidence suggests that these transport mechanisms may not exist in jejunal tissue.

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CHAPTER 2

DIFFERENTIAL AND INTERACTIVE EFFECTS OF CALCIUM CHANNEL BLOCKERS AND CHOLESTEROL CONTENT OF THE DIET ON JEJUNAL UPTAKE OF LIPIDS IN RABBITS[1]

2.1) INTRODUCTION

Pharmacologic agents that block calcium entry into cells may be chronically administered for the treatment of several disorders including angina pectoris, hypertension, supraventricular tachycardia and posthemorrhagic cerebral vasospasm (Vaghy et al, 1987). In addition, numerous accounts of the potential antiatherogenic effects of these compounds, collectively referred to as calcium channel blockers (CCBs), have been reported (Henry and Bentley, 1981; Rouleau et al, 1983; Blumlein et al, 1984; Willis et al, 1985; Fronek, 1988; Catapano et al, 1988; Sugano et al, 1986; Senaratne et al, 1991). Administering CCBs to animals fed a high cholesterol diet (HCD) reduces the formation of atherosclerotic lesions by a mechanism that is still unclear (Henry, 1985; Hof et al, 1990). Studies in humans also suggest a favourable influence of CCBs on atherosclerosis (Lichtlen et al, 1990). Several anti-atherosclerotic actions related to the blockade of calcium entry have been proposed including: inhibition of arterial smooth muscle cell proliferation and migration (Nakao et al, 1983; Betz, 1988; Jackson et al, 1989), hypotensive effects, and protection from arterial cell necrosis due to reduced "calcium overload" (Henry, 1985, 1987). Cell culture experiments have suggested that some CCBs alter metabolism of low-density lipoproteins (Ranganathan et al, 1982), enhance LDL receptor activity (Paoletti et al, 1988) or promote cholesterol ester

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hydrolase activity in lipid-rich aortic smooth muscle cells (Etingin and Hajar, 1985). It is possible that other ancillary properties of CCBs also exert an antiatherosclerotic effect (Weinstein and Heider, 1987; Hof et al, 1990).

In contrast, some groups have reported that the administration of CCBs has failed to significantly reduce atherosclerosis (Naito et al, 1984; Stender et al, 1984; Diccianni et al, 1987; Kritchevsky, 1988). These negative results, in addition to the variety of available CCBs, the diversity of dietary cholesterol:drug ratios and experimental species, as well as the various drug administration routes, have made it difficult to conclusively define the mechanism and effects of CCBs on atherosclerosis.

Using a rabbit model of atherosclerosis, we have observed a 23-44% reduction in serum total cholesterol levels in animals administered the CCB nisoldipine (N) (oral dose 1 mg/kg) simultaneously with the initiation of a HCD (Senaratne et al, 1991). In addition, aortic cholesterol content is significantly diminished by feeding N. Reductions in serum cholesterol levels have been reported by others. For example, Sugano et al (1986) reported significantly lower total and LDL plasma cholesterol levels in Japanese white rabbits fed a 1% cholesterol diet plus daily intraperitoneal injections of diltiazem (50 mg/day). In hypercholesterolemic rats, high doses of oral nicardipine (100 mg/g) reduced serum VLDL and LDL (Ohato et al, 1984). Furthermore, HDL levels were significantly increased in this model when nicardipine was given. At lower nicardipine doses (10 mg/kg) total cholesterol levels (mg/dL) were lower, but the difference did not achieve statistical significance. In Dutch-belted rabbits 2% dietary cholesterol plus oral nicardipine 40 mg/kg given twice daily resulted in a 42% reduction in the cholesterol content of the plasma VLDL/chylomicron fraction. Interestingly, however, nifedipine at the same dose did not exert this effect, suggesting that not all CCBs have the same effect on cholesterol metabolism (Willis et al, 1985). In a small group of human patients with angina pectoris, modest serum lipid

reductions and beneficial effects of verapamil (V) on LDL:HDL levels have been reported (Walldius, 1983).

In view of the ubiquitous role of calcium in the regulation of cell function, it is reasonable to speculate that long-term altered influx of calcium may have important effects in tissues other than the blood vessels involved in the atherosclerotic process. Recently, radioligand binding and short circuit current studies have suggested the presence of voltage-dependent calcium channels in rabbit ileum (Homaidan et al, 1989). The magnitude of the effects in intestine may vary with different classes of CCBs.

The effects of chronic intestinal CCB exposure on lipid uptake processes in the small intestine have not been reported. Thus, this study was undertaken to determine if the in vitro jejunal lipid uptake was affected by the administration of two different classes of CCBs, a 1,4-dihydropyridine N, nisoldipine and a phenylalkylamine V, verapamil. We wished to test the hypothesis that N modifies plasma lipid levels by way of inhibiting the intestinal uptake of long chain fatty acids and cholesterol. The results suggest that modification of the intestinal uptake of lipids does not represent the mechanism by which N may reduce serum and tissue levels of lipids in the rabbit.

2.2) METHODS

2.2.1) ANIMALS

The study was conducted using 60 male New Zealand White rabbits, 8-10 weeks old and weighing 1.5-2.0 kg. All animals were maintained on Baby Rabbit Pellets® (United Feeds, Edmonton, AB) for 1 week before being randomly assigned to control or to experimental groups. The rabbits were housed individually under the same conditions, with controlled lighting and ventilation conditions. Food intake and body weight were monitored, and water was provided ad-libitum. Blood samples were collected from the middle ear vein

for determination of serum cholesterol, triglyceride and glucose concentrations at the initiation and completion of the treatment period.

2.2.2) STUDY DESIGN

The study was designed to determine if jejunal nutrient uptake in vitro was affected by short-term (acute) intestinal exposure (6 and 36 minute) and long-term (chronic) exposure (3 week feeding) to the dihydropyridine derivative, nisoldipine (N) and to the phenylalkylamine, verapamil (V). Acute effects of N exposure were determined for the jejunal uptake of cholesterol and palmitic acid (16:0). Chronic effects of N or V were determined for cholesterol, stearic acid (18:0), oleic acid (18:1) and linoleic acid (18:2). For all studies, a previously-validated method for determining in vitro nutrient uptake in intact intestinal tissue was used (Westergaard and Dietschy, 1974).

A) Acute Experiments

In the acute experiments one group of 12 animals was maintained on chow (hereafter "low cholesterol diet" or "LCD") and a second group of 12 rabbits was fed the same pellets modified to contain 2.8% cholesterol (w/w), referred to as "high cholesterol diet" or "HCD". The cholesterol content of the LCD was not determined in this study. In a previous analysis of a similar diet the cholesterol content was determined to be 0.06%. The HCD was prepared by adding cholesterol (5-Cholesten-3β-ol; Sigma Chemical Co., St. Louis, MO) to the low cholesterol pellets. Briefly, 4 kg allotments were thoroughly mixed with 100 g of cholesterol dissolved in 500 g of ethyl ether (reagent grade; Fisher Scientific Ltd., Fair Lawn, NJ). The prepared food mixture was then spread out on trays and placed in a fume hood to dry for 48 hours. To validate this method, the cholesterol content of eight random duplicate samples of the prepared diet was estimated using a modified Folch's lipid extraction procedure (Bowyer and King, 1977; Morin, 1976) and a commercial enzymatic

colorimetric test for cholesterol determination (Boehringer Mannheim, West Germany). The cholesterol content was determined to be 2.8%±0.2 (mean±SEM).

B) Chronic Experiments

For the chronic drug experiments animals were divided into "no drug" groups including LCD (n=6 and 6) and HCD (n=6 and 6) and "drug administered" groups including LCD plus N (n=6), LCD plus V (n=6), HCD plus N (n=6) and HCD plus V (n=6).

2.2.3) DRUG DOSES AND ADMINISTRATION

All feeding treatments were for a 3 week duration. Solubilized N and V (as described below) were provided orally by syringe-feeding at daily doses of 1 mg/kg body weight and 4 mg/kg, respectively. The dose of N was selected to duplicate the model of Senaratne et al (1991) and to avoid hemodynamic effects of the drug. A dose of 1 mg/kg N effectively reduces atherosclerosis and is also well below the dose of 20 mg/day which was previously shown to decrease mean arterial pressure in cholesterol-fed New Zealand white rabbits (Fronek, 1988). The V dose of 4 mg/kg was selected to approximate clinical doses used in humans (240-360 mg/day on average for a 70 kg body weight adult) (McTavish and Sorkin, 1989), rather than the higher doses used in most reported animal studies (Rouleau et al, 1983; Blumlein et al, 1984). This dose is also not associated with hemodynamic effects (Blumlein et al, 1984).

Preliminary studies with the administration vehicle used in this study (i.e. introduction of aqueous solution by a syringe into the mouth) were shown to be without influence upon the effect of cholesterol feeding (unpublished observations, Kappagoda, 1989). Thus, a control vehicle was not used in the present study.

2.2.4) CHEMICALS

Unlabelled cholesterol and fatty acids, were all >99% pure as supplied by Sigma Chemical Co. (St. Louis, MO). Taurodeoxycholic acid (TDC) was obtained from Calbiochem

Corp. (La Jolla, CA). The compound used to measure the intestinal adherent mucosal fluid volume, [^{3}H]inulin (molecular weight approximately 5000), was obtained from Amersham Canada Ltd. (Oakville, ON). The suppliers of the [^{14}C]-labelled compounds include cholesterol from Amersham Canada Ltd. (Oakville, ON), and palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1) linoleic acid (18:2) from New England Nuclear (Boston, MA). All other compounds used were of reagent grade as supplied by Fisher Scientific Ltd. (Fair Lawn, NJ).

N in powdered form was provided as a gift from Miles Pharmaceutical Ltd. (New Haven, CT). The drug was solubilized in 3.5 mL of 95% ethanol (<1.5% of the final administration volume) and distilled deionized water for oral administration. V-HCl was obtained from Sigma Chemical Co. (St. Louis, MO), and was solubilized in distilled deionized water. The drugs were concentrated in the solution so that the average quantity of fluid provided daily to achieve the required dose was 0.35 mL. The mean quantity of ethanol being received each day in the N administered rabbits was 5.4 uL. This is well below the amount of chronic ethanol (approximately 26 mL/day for 6-7 weeks) associated with altered uptake of lipids and glucose in rabbit jejunum (Thomson, 1984a).

2.2.5) PREPARATION OF INCUBATION SOLUTIONS

Test solutions containing cholesterol or fatty acids were prepared as described previously (Westergaard and Dietschy, 1976). Briefly, the solutions were prepared by adding appropriate amounts of both the [^{14}C]-labelled and unlabelled test compound to an incubation beaker and dissolving these in 2 mL of chloroform/methanol (2:1, v/v). The chloroform/methanol phase was evaporated under nitrogen and 150 mL of taurodeoxycholic acid (TDC) solution in Krebs-bicarbonate buffer (pH 7.4) was added. Solutions containing cholesterol were sonicated for 15 minutes at 40-42°C in a Branson 1200 sonicator. Appropriate amounts of [^{3}H]inulin were added and all solutions were stirred at 37°C with

a magnetic bar for 3 hours. All fatty acid concentrations in the micellar solutions were 0.1 mM in 20 mM TDC. Cholesterol concentrations of 0.1 mM in both 10 and 20 mM TDC were used.

For the acute studies, test solutions were prepared as described above with the addition of N in concentrations ranging from 10^{-8}M to 10^{-4}M. All preparation and experimentation with N was conducted in a darkened room to avoid possible photodegradation of the drug.

2.2.6) TISSUE PREPARATION

The rabbits were anesthetized with pentobarbitone sodium (25 mg/kg), and the jejunal segment was quickly removed and rinsed with 150 mL of ice cold saline. The segments were opened along the mesenteric border, rinsed gently with 50 mL of saline and were cut into several smaller sections of approximately 1.5 cm length. These sections were then mounted as flat sheets in incubation chambers clamped between two plastic plates to allow exposure of the mucosal surface to the incubation medium through an aperture exactly 1.4 cm in diameter. The mounted chambers were placed in beakers containing 150 mL of oxygenated Krebs-bicarbonate buffer at 37°C for a preincubation period of 30 minutes. All solutions were mixed at identical rates with circular magnetic bars to reduce the resistance of the intestinal unstirred water layer. This allowed for the better assessment of the transport properties of the brush border membrane (Westergaard and Dietschy, 1974). The stirring rate in this study was adjusted precisely to 600 revolutions per minute using a strobe light. For acute exposure experiments half of the tissues were preincubated in Krebs-bicarbonate containing N ranging from 10^{-8}M to 10^{-4}M for 30 minutes, while the other half were preincubated in the drug free Krebs solution for the same time period.

2.2.7) DETERMINATION OF RATES OF UPTAKE

After the 30 minute preincubation period, the transport chambers were transferred to beakers containing the radiolabelled test solutions for a strictly timed incubation period of 6 minutes. The experiment was then terminated by removing the chamber and quickly rinsing the mounted tissue in cold saline. The tissue segments were cut out from the transport chamber using a circular steel punch, were placed on glass slides and dried overnight at 55°C in a drying oven. After determining the dry intestinal weight, the tissue pieces were transferred to scintillation vials and were saponified in 0.4 N sodium hydroxide. Scintillation fluid was added and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes. The rate of uptake was calculated after correcting the total tissue [^{14}C]-radioactivity for the mass of probe molecule present in the adherent mucosal fluid.

The rate of lipid uptake was expressed as the nmol of probe molecule taken up into the mucosa per 100 mg dry weight of mucosa (nmol/100 mg mucosa·min^{-1}). The weight of the mucosa as well as the ratio of mucosa:submucosa was determined by scraping the mucosal layer from the underlying layer with a glass slide. Uptake rates were also calculated as nmol of probe taken up per minute per unit serosal surface area.

The values obtained for the different groups of animals are reported as the mean±SEM of the results from 6-12 animals in each group.

2.2.8) SERUM CHOLESTEROL AND TRIGLYCERIDE DETERMINATION

The cholesterol, triglyceride, and glucose measurements in the serum were determined by the use of an automated system (Multistat III, Instrumentation Laboratories, Lexington, KY) which incorporated the methods of Allain et al (1974) and Pinter et al (1967).

2.2.9) MORPHOLOGY

The morphology of the jejunal tissue was assessed as previously described (Ecknauer et al, 1982; Keelan et al, 1985a, 1985b). Morphological measurements were done on 1 cm sections of tissue fixed in Bouin's solution, dehydrated and embedded in paraffin. Sections were cut at a thickness of 5 μm for light microscopy, mounted on glass slides and stained with hematoxylin and eosin using standard techniques. A Scopion projecting microscope was used to project the tissue sections at 1000-fold magnification. Measurements of villus height, villus width at 1/2 height, villus bottom width and crypt depth were obtained from vertical tissue sections. Measurement of villus thickness (a second dimension of villus width) was obtained from transverse tissue sections.

The villus surface area (VSA, μm^2/villus) was calculated according to the following formula:

$$VSA = (2xMxH)+(2xM-A)xD+(2xDx(((A-M^2)+(H)^2))^{0.5}x1000$$

where H = villus height; M = villus width at 1/2 height; A = villus bottom width; D = villus thickness at 1/2 height. To estimate villus density, the number of villi per millimeter were measured in longitudinal and horizontal cross-sections, and were then multiplied together to obtain the number of villi per mm^2 serosa. At least 10 villi were assessed per section. The mucosal surface area (MSA, mm^2/mm^2 serosa) was calculated from the formula:

MSA = number of villi/mm^2 serosa x VSA (mm^2/villus)

2.2.10) STATISTICAL ANALYSIS

The statistical significance of the difference between means was determined using a two-way analysis of variance procedure followed by the Student-Neumann Kuels multiple range test and unpaired t-tests to determine differences between individual treatment means.

2.3) RESULTS

2.3.1) ANIMAL CHARACTERISTICS

The oral administration of nisoldipine (N) and verapamil (V) was well tolerated, and there were no deaths or significant adverse events during the 3 week course of the study. All rabbits consumed the same amount of food, with the exception of the higher food intake in animals fed the low cholesterol diet (LCD) plus N as compared with LCD, yet body weight gain was equal among all groups (Tables 2-1 - 2-3).

As expected, serum cholesterol and triglyceride concentrations were higher in animals fed high cholesterol diet (HCD) as compared with LCD. Adding V to LCD or HCD did not significantly affect these lipid levels. However, rabbits fed HCD plus N (Table 2-2) had lower serum cholesterol levels (880.6±93.6 mg/dL in HCD versus 587.5±22.6 mg/dL in HCD plus N). Although serum triglyceride levels were lower in HCD plus V versus HCD (Table 2-3), this difference was not statistically significant. Serum glucose levels were similar in LCD or HCD with or without N or V (Tables 2-2 and 2-3).

2.3.2) INTESTINAL MORPHOLOGY

Adding N or V to HCD lowered the dry weight of the jejunum (HCD plus N or HCD plus V versus HCD), but adding calcium channel blockers (CCBs) to LCD did not affect the weight of the jejunum (Table 2-4). Because of these differences in jejunal weight, and the lower percentage of the intestinal wall comprised of mucosa in HCD plus V, rates of uptake were expressed on the basis of the dry weight of the mucosa rather than the dry weight of the entire wall of the jejunum.

The villus height was significantly increased in the HCD plus N and HCD plus V groups as compared to the HCD animals not given CCBs, but because of adjustments in the dimensions of the width and thickness of the villus, the mucosal surface area was not altered among any of the animal groups.

2.3.3) LIPID UPTAKE

A) Acute Studies

The jejunal uptake of 0.1 mM cholesterol and 0.1 mM palmitic acid (16:0) in 20 mM taurodeoxycholic acid (TDC) was unaffected by short-term exposure to increasing concentrations of N (Tables 2-5 and 2-6). The lack of effect was observed in rabbits fed either LCD or HCD.

B) Chronic Studies

The feeding of N for 3 weeks had differential effects upon the jejunal uptake of cholesterol depending upon the ratio of TDC:cholesterol in the test solution (Table 2-7). When 10 mM TDC was used to solubilize the 0.1 mM cholesterol, uptake was increased in both LCD plus N and HCD plus N, as compared to LCD or HCD. In contrast, when 20 mM TDC concentration was used to solubilize cholesterol, cholesterol uptake was reduced in LCD plus N as compared with LCD. A different pattern of effects was noted when V was added to LCD or HCD: V increased cholesterol uptake in LCD (LCD plus V versus LCD), yet decreased cholesterol uptake in HCD (HCD plus V versus HCD) (Table 2-8). Thus, adding N or V to LCD increased cholesterol uptake, V lowered cholesterol uptake in HCD yet N enhanced uptake in HCD. Both N and V increased the jejunal uptake of stearic acid (18:0) in LCD. N had no effect on fatty acid uptake in HCD, whereas V lowered the uptake of stearic and linoleic acids yet increased the uptake of oleic acid (Tables 2-7 and 2-8).

2.4) DISCUSSION

Diabetes, abdominal irradiation, bowel resection and changes in dietary lipids alter the functional characteristics of the intestine and therefore affect rates of nutrient absorption (Thomson et al, 1990; Thomson et al, 1989). The results of this study indicate that lipid uptake into the jejunum is differentially affected by two classes of calcium channel blockers

(CCBs). In addition, the direction of these effects is influenced by the level of cholesterol in the diet. This effect of nisoldipine (N) or verapamil (V) is likely due to an adaptive process in the intestine occurring over the 3 weeks of feeding, since N did not affect cholesterol or palmitic acid uptake when added directly to the incubation medium (Tables 2-5 and 2-6).

Interestingly, the ratio of cholesterol:taurodeoxycholic acid (TDC) used in the solubilization of cholesterol affected the direction of uptake response in animals administered N but not V. Increasing the ratio of TDC:cholesterol has previously been shown to lower in vitro cholesterol uptake, possibly due to reduced partitioning from the micelle to the monomeric phase adjacent to the brush border membrane (Westergaard and Dietschy, 1976; Thomson et al, 1983). It is possible that the effects of N plus LCD in achieving a reduction in cholesterol uptake when solubilized in 20 mM TDC, yet enhanced cholesterol uptake with 10 mM TDC (Table 2-7), may have been due to an effect of N on partitioning of cholesterol from the TDC micelle. This possibility is strengthened by the observation of a variable effect of N on cholesterol uptake in HCD. The directionally similar effects of V on cholesterol uptake in LCD and HCD with 10 and 20 mM TDC may be due to greater intestinal binding of V (Homaidan et al, 1989) or to variable degrees of tissue specificity among the CCBs (Katz, 1986; Hof et al, 1990).

A similar pattern was identified for the uptake of stearic acid; enhanced uptake of this fatty acid in animals fed N or V while on a LCD, but reduced uptake in animals given V while on a HCD. However, not all of the fatty acids studied were influenced in the same manner (Tables 2-7 and 2-8). Heterogeneity of the uptake of lipids has been suggested from a theoretical perspective (Winne, 1978), and the variable effect of N or V on the mono- or polyunsaturated as compared with the saturated fatty acids would support this view. In addition, however, there were qualitative differences in the effect of N and V on the uptake

of fatty acids. Thus, although the mechanisms of the effect of CCBs on lipid uptake have not been established in this study, the mechanisms appear to vary between these two different classes of CCBs.

The differential effects of N and V on the intestine were particularly apparent in the animals fed HCD, and this differential effect has been noted in other tissues. In cholesterol-enriched but not in control perfused rabbit aorta, V and diltiazem have been shown to antagonize the effect of norepinephrine-stimulated calcium influx (Bialecki and Tulenko, 1989). These authors suggested that cholesterol enrichment of the plasma membrane may have exposed sites that were otherwise unavailable for binding to these CCBs. Since different CCBs appear to bind to different sites on calcium channel proteins (Glossman et al, 1985), it may be speculated that in our cholesterol-fed model alterations in enterocyte membrane lipid composition achieved by feeding cholesterol (HCD versus LCD) (Brasitus, 1987) may affect the binding site for V differently from that of N.

Several possible mechanisms of intestinal adaptation to various stimuli have been suggested (Thomson et al, 1989, 1990), including changes in the effective resistance of the unstirred water layer (UWL) or the lipid composition of the brush border membrane (Keelan, 1985a, 1985b, 1985c; Brasitus and Schacter, 1985; Stenson et al, 1988; Thomson et al, 1984b, 1990). It has been reported that the effective resistance of the UWL was decreased in the cholesterol fed animals, and this would be expected to be associated with a higher uptake of lipids (Thomson, 1982a; Thomson et al, 1987). In this study we did not assess the effective resistance of the UWL. However, we did observe a reduced uptake of most lipids in HCD plus V and no reductions in HCD plus N (Tables 2-7 and 2-8). Thus, it is unlikely that the effect of N or V on cholesterol or fatty acid uptake was due to an affect on UWL resistance. The movement of lipids through the intestinal brush border membrane is believed to be mainly a passive process, although a carrier-mediated component has been

suggested for fatty acids and possibly cholesterol (Stremmel, 1985, 1988). In vitro CCBs have been associated with the de novo synthesis of LDL receptors, suggesting their potential for synthetic activity of membrane-bound proteins (Paoletti et al, 1988). We have not assessed the effect of N or V on brush border membrane lipid composition or membrane fatty acid binding protein.

In spite of a significant increase in villus height in both the HCD plus N and HCD plus V animals, there were no variations in mucosal surface area between drug administered versus control groups (Table 2-4). Two dimensional measurements (villus height and width) of mucosal surface area do not necessarily provide reliable assessments of surface area. For example, in this study the villus density (number of villi/mm^2 serosa) decreased in HCD plus N and HCD plus V, countering the increase in villus height, so that the mucosal surface area was unchanged. Thus, the results of this study cannot be explained on the basis of variations in the intestinal mucosal surface area, nor can the results be explained on the basis of any differences in the animals' body weight gain or food intake (Tables 2-1 - 2-4).

A lack of correlation between mucosal surface area and nutrient transport function has been reported in other studies (Keelan et al, 1985a, 1985c). The functional surface area for uptake across the villus may not be the same for all nutrients (Chang et al, 1987; Cheeseman, 1986; Maenz and Cheeseman, 1986; Winne, 1978), so that changes in total villus a mucosal surface areas may not necessarily be associated with changes in the functional surface area used for nutrient uptake. Furthermore, the lack of a change in villus surface area in an experimental setting does not necessarily signify the lack of change of the functional villus surface area.

Polyamines (putrescine, spermidine and spermine) and their rate-regulating enzyme ornithine decarboxylase (ODC) may be important in cell growth, replication and differentiation (Pegg and McCann, 1982). In an osteogenic sarcoma cell line (UMR 106-01)

V dose-dependently reduced basal and stimulated ODC activity (Langdon, 1984). The results of this study suggest a role for calcium in basal ODC regulation, and a calcium, cAMP-dependent mediation of stimulated ODC activity. It is unknown what the effects of chronic CCB feeding on intestinal ODC activity might be. It has been observed that ODC levels are higher in villous than crypt cells, implying polyamine involvement in mature or differentiating intestinal cells (Baylin et al, 1978; Sepulveda et al, 1982). Thus, it might be speculated that altered ODC levels would affect the functional portion of the villus with resulting effects on nutrient uptake. If cell migration or turnover is affected by polyamines, then reduced ODC levels possibly achieved by feeding V or N might allow the enterocytes remaining on the villus to mature and to acquire greater transport potential. Static measurements of mucosal surface area do not indicate which portion of the villus and which particular functional component may be affected by feeding cholesterol or CCBs, nor do they provide information regarding cell proliferation, differentiation or migration rates. Thus, it is possible that the CCBs resulted in a change in the dynamic morphology of the intestine and thereby influenced lipid uptake. This speculation awaits direct testing.

Effects of CCBs on intracellular lipid metabolizing enzymes (such as fatty acid desaturase, acylation and deacylation enzymes, or the cholesterol and phospholipid metabolizing enzymes) could alter brush border membrane lipid composition, thereby resulting in altered permeability properties of the membrane (Garg et al, 1988; Brasitus and Schacter, 1982). The enterocyte microsomes responsible for the desaturation and elongation of fatty acids may be affected by dietary lipids (unpublished observations; Garg et al, 1989), but it is unknown if calcium or CCBs play a role in their activity. Changes in the activity of cholesterol esterifying or synthesizing enzymes could also potentially affect the uptake of these lipids. A calcium, calmodulin dependent kinase has been suggested to play a role in the short-term regulation (via phosphorylation and concomitant inactivation) of HMG-CoA

reductase, the rate-limiting enzyme for cholesterol synthesis (Beg et al 1987a, 1987b). Reduced calcium flux might upregulate the expressed activity of HMG-CoA reductase by promoting the dephosphorylation of the enzyme. However, if a calcium-dependent step was affected by the presence of CCBs in the enterocyte, it would be anticipated that both classes of drug would affect intracellular synthesis of cholesterol and thereby would have directionally similar effects on the brush border membrane composition of cholesterol (Brasitus and Schacter, 1982). Calcium may play a direct role in modulating membrane lipids by binding to anionic sites of the membrane bilayer. However, in rat brush border membrane vesicles intraluminal calcium decreased the fluidity by alterations in membrane sphingomyelin content by unknown, presumably indirect mechanisms (Dudeja et al, 1987). Calcium-mediated alterations in lipid composition have also been observed in hepatocytes (Livingstone and Schacter, 1980).

In cultured macrophages CCBs inhibit cholesterol esterification (Daugherty, 1987). The mechanism for this action has not been defined, but it is apparently independent of the calcium-blockade effect, and could involve a direct effect on the ACAT reaction in this tissue. Furthermore, not all classes of CCB drugs exhibited this inhibitory effect. It is unknown whether a similar effect might apply to the intestine, but clearly alterations in the activity of ACAT also influence the intestinal uptake of cholesterol (Tso, 1985). It is interesting to speculate that the upregulated state of ACAT following cholesterol feeding as well as possible differential effects of CCBs on this enzyme could account for some of the variable effects noted between CCBs and diet effects.

CCBs affect short circuit current (Isc) measured across the ileal tissue, with V having a quantitatively greater effect than other classes of CCBs (Homaidan et al, 1989). This change in Isc represents the net effect of calcium channel blockade on the flux of anions and cations from the mucosa to the serosa, and from the serosa to the mucosa. In the presence

of CCBs, equally enhanced mucosal-to-serosal movement of sodium and chloride exceed serosal-to-mucosal movement of these ions. Residual fluxes (likely bicarbonate ions) and Isc are decreased (Donowitz and Asarkof, 1982). Removal of sodium from the bulk phase reduces the uptake of fatty acids (Thomson, 1982b; Stremmel, 1988), but it is unknown what effect an enhanced mucosal-to-serosal flux of sodium might have on lipid uptake. In cardiac myocytes and peritoneal mast cells, reductions in extracellular calcium increase sodium influx and permeability; these effects are associated with an enhancement of sodium pump (Na^+/K^+-ATPase) activity (Hagane et al, 1989; Knudsen and Johansen, 1989). It is unknown whether calcium plays a role in the physiological control of the intestinal basolateral membrane sodium pump, or whether CCBs exert an important influence. There appear to be at least two isoforms of this ATPase in the intestine (Fedorak, 1990) which could also be differentially affected by cholesterol feeding or by different classes of CCBs. CCBs in the serosal medium bathing jejunal tissue reduces the magnitude of glucose stimulated Isc in LCD but not HCD animals (unpublished observations, Hyson et al, 1991). However, the variable effect of V on the uptake of the three fatty acids examined in this study suggest that the mechanism is not likely explained solely on the basis of altered effects on sodium gradients across the enterocyte.

Studies in rabbit ileal brush border (Emmer et al, 1989; Rood et al, 1988) and chicken enterocytes (Semrad and Chang, 1987), have suggested a role for calcium in regulating the activity of the intestine Na/H^+ antiporter. Protein kinase C and cAMP mediated inhibition of the activity of the Na/H^+ antiporter was associated with increased cell surface pH in rat jejunal villus cells (Shimada and Hoshi, 1988). Although calcium ionophores did not cause a similar inhibition, it is unknown if altered intracellular calcium levels were achieved; thus a role for calcium was not ruled out. It is unknown if CCBs would enhance Na/H^+ exchange. However, it would be expected that increased activity of the

antiporter would increase the pH of the acidic microclimate adjacent to the mucosal membrane (Lucas et al, 1975). Since fatty acids would be protonated at a low pH, their solubility in micelles would be reduced and partitioning into the lipid membrane would be enhanced (Shiau, 1987). In this study both N and V enhanced stearic acid uptake in LCD, and V increased the uptake of oleic acid.

Our initial interest of the possible effect of CCBs on intestinal lipid uptake was stimulated by the observation of the cholesterol-lowering effects (both serum and aortic) in animals fed N simultaneously with a HCD (Senaratne et al, 1991). This cholesterol-lowering effect of N was clearly not due to a decline in the intestinal uptake of lipids (Table 2-7). While V reduced the uptake of lipids in HCD (Table 2-8), it did not reduce levels of cholesterol in serum (Table 2-3). Thus, it is likely that the effect of N on cholesterol concentration lies beyond the intestine.

TABLE 2-1: Characteristics of animals used in the study of acute drug exposure.

	LCD	HCD
WEIGHT GAIN (kg/3 weeks)	0.60±0.05	0.54±0.04
TOTAL FOOD CONSUMED (g/3 weeks)	2313±172	2364±154
CHOLESTEROL INTAKE (g/3 weeks)	n/a	59.10±3.84
SERUM PARAMETERS (mg/dL)		
Cholesterol (0)	85.4±5.0	79.7±5.6
Cholesterol (3)	77.9±10.7	841.5±132.5[a]
Triglyceride (0)	80.0±7.5	109.3±16.4
Triglyceride (3)	81.2±13.5	132.6±16.9
Glucose (0)	161.0±21.6	175.1±31.4
Glucose (3)	112.7±16.2	135.6±7.0

Values are mean±SE; LCD = low cholesterol diet; HCD = high cholesterol diet; (0) = value at initiation of study; (3) = value after 3 week feeding; n/a = not applicable

[a] $p<0.05$ LCD versus HCD

TABLE 2-2: Characteristics of nisoldipine (N)-administered and control animals.

	LCD	LCD+N	HCD	HCD+N
WEIGHT GAIN (kg/3 weeks)	0.64±0.03	0.63±0.05	0.64±0.05	0.57±0.05
TOTAL FOOD CONSUMED (g/3 weeks)	1614±54	1917±119[a]	1977±104	1805±106
CHOLESTEROL INTAKE (g/3 weeks)	n/a	n/a	49.41±2.58	45.09±2.65
SERUM PARAMETERS (mg/dL)				
Cholesterol (0)	103.4±9.5	107.3±6.0	108.9±9.6	105.3±5.8
Cholesterol (3)	73.1±4.1	63.9±3.3	880.6±93.6[b]	587.5±22.6[b,c]
Triglyceride (0)	169.9±53.7	123.0±24.8	156.4±24.9	137.0±21.5
Triglyceride (3)	91.4±14.1	87.1±9.4	126.7±15.8	139.8±31.9
Glucose (0)	154.7±9.4	165.4±7.6	146.8±5.1	153.4±4.9
Glucose (3)	161.6±12.5	147.1±14.1	144.7±6.1	139.6±8.1

Values are mean±SE; LCD = low cholesterol diet; LCD+N = low cholesterol diet plus nisoldipine; HCD = high cholesterol diet; HCD+N = high cholesterol diet plus nisoldipine; (0) = value at initiation of study; (3) = value after 3 week feeding; n/a = not applicable

[a] $p<0.05$ LCD versus LCD+N
[b] $p<0.05$ HCD, HCD+N versus LCD, LCD+N
[c] $p<0.05$ HCD versus HCD+N

TABLE 2-3: Characteristics of verapamil (V)-administered and control animals.

	LCD	LCD+V	HCD	HCD+V
WEIGHT GAIN (kg/3 weeks)	0.72±0.04	0.64±0.06	0.65±0.09	0.60±0.08
TOTAL FOOD CONSUMED (g/3 weeks)	2421±153	1946±77	2287±71	2427±254
CHOLESTEROL INTAKE (g/3 weeks)	n/a	n/a	57.15±1.76	59.82±7.04
SERUM PARAMETERS (mg/dL)				
Cholesterol (0)	62.4±3.9	100.4±9.3	103.1±5.9	71.5±8.1
Cholesterol (3)	57.3±5.9	70.4±6.2	1010.6±295.2[a]	992.1±217.8[a]
Triglyceride (0)	91.9±18.1	121.2±27.8	132.1±27.3	95.1±15.1
Triglyceride (3)	73.4±16.1	56.2±15.5	156.6±44.9[a]	108.6±27.2[a]
Glucose (0)	147.0±11.2	139.5±5.4	213.3±37.8	191.8±29.1
Glucose (3)	154.3±8.9	145.8±10.3	156.2±5.4	155.8±16.7

Values are mean±SE; LCD = low cholesterol diet; LCD+V = low cholesterol diet plus verapamil; HCD = high cholesterol diet; HCD+V = high cholesterol diet plus verapamil; (0) = value at initiation of study; (3) = value after 3 week feeding; n/a = not applicable

[a] $p<0.05$ HCD, HCD+V versus LCD, LCD+V

TABLE 2-4: Jejunal characteristics and morphology of study animals.

	LCD	HCD	LCD+N	HCD+N	LCD+V	HCD+V
Dry weight mg/unit serosal surface area	35.7±2.3	39.2±1.5	31.6±1.2	31.7±1.8[b]	34.1±1.3	32.5±1.6[c]
% of intestinal wall comprised of mucosa	84.1±1.8	83.6±1.1	78.4±3.0	82.9±1.9	78.9±2.1	72.2±1.2[c]
Crypt depth (μm)	50±2.9	43±2.5	51±2.6	69±3.0[b]	44±2.7	44±2.1
Villus height (μm)	603±29	599±24	573±25	717±33[b]	680±42	752±50[c]
Villus width at half height (μm)	94±4	126±6	98±4	158±8	114±6	124±6
Villus bottom width (μm)	114±7	135±5	122±5	166±6	127±8	134±7
Villus thickness (μm)	431±28	210±12	327±30	390±50	340±25	267±11
Villus surface area (μm^2/villus)	666±32	428±18	511±23	846±45	651±37	622±47
No. of villi/mm^2 serosa	21.3±1.4	35.6±1.2	25.3±1.0[a]	15.7±0.6[b]	24.2±1.7	28.8±1.5[c]
Mucosal surface area (mm^2/mm^2 serosa)	14.0±1.0	15.2±0.9	13.0±0.9	13.2±0.8	15.81±1.5	17.8±1.5

Values are mean±SE; LCD = low cholesterol diet; LCD+N = low cholesterol diet plus nisoldipine; LCD+V = low cholesterol diet plus verapamil; HCD = high cholesterol diet; HCD+N = high cholesterol diet plus nisoldipine; HCD+V = high cholesterol diet plus verapamil

[a] ($p<0.05$) LCD versus LCD+N
[b] ($p<0.05$) HCD versus HCD+N
[c] ($p<0.05$) HCD versus HCD+V

TABLE 2-5: Effect of acute tissue exposure to nisoldipine (N) on jejunal cholesterol uptake.

Incubation Concentration (M) of Nisoldipine	6 Minute Exposure to N		36 Minute Exposure to N	
	LCD	HCD	LCD	HCD
No Drug	0.09±0.01	0.14±0.02	0.07±0.01	0.05±0.01
10^{-8}	0.08±0.01	0.10±0.01	0.12±0.02	0.09±0.02
10^{-7}	0.11±0.01	0.14±0.02	0.11±0.02	0.08±0.01
10^{-6}	0.09±0.01	0.08±0.02	0.14±0.02	0.07±0.01
10^{-5}	0.07±0.01	0.09±0.02	0.08±0.01	0.09±0.01
10^{-4}	0.09±0.01	0.10±0.01	0.09±0.01	0.08±0.01

Values are mean±SE; LCD = low cholesterol diet; HCD = high cholesterol diet The concentration of cholesterol was 0.1 mM in 20 mM taurodeoxycholic acid. The rate of uptake was expressed as nmol/100 mg mucosa·min^{-1}.

TABLE 2-6: Effect of acute tissue exposure to nisoldipine (N) on jejunal palmitic acid uptake.

Incubation Concentration (M) of Nisoldipine	6 Minute Exposure to N		36 Minute Exposure to N	
	LCD	HCD	LCD	HCD
10^{-8}	0.81±0.16	0.46±0.05	0.27±0.05	0.32±0.12
10^{-7}	0.77±0.23	0.41±0.13	0.49±0.08	0.34±0.06
10^{-6}	0.29±0.08	0.21±0.05	0.24±0.05	0.47±0.19
10^{-5}	0.39±0.09	0.42±0.09	0.22±0.03	0.31±0.06
10^{-4}	0.32±0.07	0.24±0.06	0.33±0.05	0.27±0.05

Values are mean±SE; LCD = low cholesterol diet; HCD = high cholesterol diet
The concentration of palmitic acid was 0.1 mM in 20 mM taurodeoxycholic acid. The rate of uptake was expressed as nmol/100 mg mucosa·min^{-1}.

TABLE 2-7: Effect of chronic oral nisoldipine (N) intake (1 mg/kg·day^{-1}) on jejunal uptake of cholesterol and fatty acid.

SUBSTRATE	LCD	LCD+N	HCD	HCD+N
Cholesterol[1]	0.16±0.03	0.08±0.02[a]	0.13±0.02	0.10±0.01
Cholesterol[2]	0.30±0.06	1.01±0.10[a]	0.13±0.02	1.31±0.13[b]
Stearic Acid (18:0)	0.30±0.04	0.56±0.11[a]	0.30±0.04	0.46±0.04
Oleic Acid (18:1)	0.30±0.04	0.21±0.05	0.24±0.05	0.29±0.05
Linoleic Acid (18:2)	0.31±0.03	0.32±0.06	0.42±0.10	0.59±0.08

Values are mean±SE; LCD = low cholesterol diet; LCD+N = low cholesterol diet plus nisoldipine; HCD = high cholesterol diet; HCD+N = high cholesterol diet plus nisoldipine. The concentration of cholesterol was either (0.1 mM) in 20 mM taurodeoxycholic acid (cholesterol[1]) or 0.1 mM in 10 mM taurodeoxycholic acid (cholesterol[2]). The concentration of the fatty acids was 0.1 mM in 20 mM taurodeoxycholic acid. The rate of uptake was expressed as nmol/100 mg mucosa·min^{-1}.

[a] $p<0.05$ LCD versus LCD+N
[b] $p<0.05$ HCD versus HCD+N

TABLE 2-8: Effect of chronic oral verapamil intake (4 mg/kg·day^{-1}) on jejunal uptake of cholesterol and fatty acid.

SUBSTRATE	LCD	LCD+V	HCD	HCD+V
Cholesterol[1]	0.35±0.03	0.94±0.11[a]	0.97±0.12	0.36±0.04[b]
Cholesterol[2]	0.53±0.05	1.08±0.15[a]	0.95±0.07	0.41±0.04[b]
Stearic Acid (18:0)	0.89±0.07	1.29±0.11[a]	1.60±0.11	0.99±0.05[b]
Oleic Acid (18:1)	1.41±0.17	1.01±0.09	1.65±0.11	2.43±0.29[b]
Linoleic Acid (18:2)	1.21±0.12	1.12±0.10	1.95±0.21	1.45±0.12[b]

Values are mean±SE; LCD = low cholesterol diet; LCD+V = low cholesterol diet plus verapamil; HCD = high cholesterol diet; HCD+V = high cholesterol diet plus verapamil The concentration of cholesterol was either 0.1 mM in 20 mM taurodeoxycholic acid (cholesterol[1]) or 0.1 mM in 10 mM taurodeoxycholic acid (cholesterol[2]). The concentration of the fatty acids was 0.1 mM in 20 mM taurodeoxycholic acid. The rate of uptake was expressed as nmol/100 mg mucosa·min^{-1}.

[a] $p<0.05$ LCD versus LCD+V
[b] $p<0.05$ HCD versus HCD+V

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CHAPTER 3

DIFFERENTIAL AND INTERACTIVE EFFECTS OF CALCIUM CHANNEL BLOCKERS ON JEJUNAL UPTAKE OF HEXOSES AND SHORT CIRCUIT CURRENT IN RABBITS[1]

3.1) INTRODUCTION

A role for free cytosolic calcium in active intestinal electrolyte secretion (Bolton and Field 1977; Frizzell, 1977) and absorption (Donowitz and Asarkof, 1982; Donowitz et al, 1985) has been demonstrated. In general, experimental manoeuvres aimed at increasing intracellular calcium levels (e.g. increased extracellular calcium concentration and the use of calcium ionophores) inhibit active sodium and chloride absorption and/or stimulate active chloride secretion (Bolton and Field, 1977; Frizzell, 1977; Donowitz and Asarkof, 1982; Donowitz, 1983). Alternatively, in rabbit ileum, rat jejunum and rat colon, measures to lower cytosolic calcium have been shown to stimulate electrolyte and water absorption. These effects have been demonstrated by reducing calcium in tissue bathing media or by adding EGTA or calcium channel blocking drugs (CCBs) to solutions bathing the tissues (Hubel and Callanan, 1980; Donowitz and Asarkof, 1982; Donowitz et al, 1985).

The response of a tissue to CCBs has commonly been used to imply the existence of voltage-dependent calcium channels. Thus, several studies have suggested the existence of gated calcium entry mechanisms in intestinal tissue (Donowitz, 1983; Donowitz et al, 1985). This argument has recently been strengthened by short circuit studies (Isc) of rabbit ileum (Homaidan et al, 1989). In these studies, derivatives of the three major classes of CCBs (phenylalkylamines, dihydropyridines and benzodiazepines) influenced Isc and

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therefore intestinal ion fluxes. Differing magnitudes of effects between classes of CCBs, as well as radioligand binding studies, suggested the possibility of at least two different calcium channels or binding sites in rabbit ileum. The putative channels appeared to be localized on the serosal side of the ileum, although this is not necessarily the case for the rat jejunum (Markowitz et al, 1985) or colon (Donowitz et al, 1985).

There is only limited information of the role of calcium and calcium channels on nutrient uptake into the intestine. Fondacaro and Madden (1984) used rat jejunal brush border membrane vesicles to study the acute effect of increased intravesicular calcium concentration on [^{14}C]-glucose uptake: glucose as well as valine uptake was significantly inhibited in calcium-loaded vesicles and the authors suggested that intravesicular calcium may suppress sodium-dependent solute transport in the intestine. In view of the importance of sodium electrochemical gradients in the transport of many nutrients, it might be anticipated that these sodium-dependent transport processes would be influenced by the CCB effects on sodium permeability. Nonetheless, in acute rabbit ileal Isc studies, glucose-stimulated sodium fluxes do not appear to be affected by the presence of various CCBs in the bathing medium (Hubel and Callanan, 1980; Donowitz and Asarkof, 1982). The acute effect of CCBs on jejunal uptake of actively transported nutrients is unknown, nor is it known whether chronic administration of CCBs alter intestinal nutrient uptake.

We have recently observed that the chronic administration of nisoldipine (N) and verapamil (V) influences the uptake of cholesterol and long chain fatty acids in rabbit jejunum (unpublished observations, Hyson et al, 1991). N and V exert differential effects, and the direction of these effects is influenced by the cholesterol content of the animals' diet. Accordingly, we studied the acute and chronic effects of N and V on the active and passive jejunal in vitro uptake of glucose and galactose in rabbits fed a low or high (2.8%) cholesterol diet.

3.2) METHODS

3.2.1) ANIMALS

The study was conducted using 77 male New Zealand White rabbits, 8-10 weeks old and weighing 1.5-2.0 kg. All animals were maintained on Baby Rabbit Pellets® (United Feeds, Edmonton, AB) for 1 week before being randomly assigned to control or to experimental groups. The rabbits were housed individually under the same conditions, with controlled lighting and ventilation conditions. Food intake and body weight were monitored and water was provided ad-libitum. Blood samples were collected from the middle ear vein for determination of serum cholesterol, triglyceride, and glucose concentrations at the initiation and completion of the treatment period.

3.2.2) STUDY DESIGN

The study was designed to determine if jejunal nutrient uptake in vitro was affected by short-term (acute) intestinal exposure (6 and 36 minute) and long-term (chronic) exposure (3 week feeding) to the dihydropyridine derivative, nisoldipine (N), and to the phenylalkylamine, verapamil (V). Acute studies were conducted to determine the effect of N and V on the jejunal uptake of D-glucose. Chronic studies were used to determine the effect of these drugs on uptake of D-glucose, galactose and L-glucose. For all studies, a previously-validated method for determining in vitro nutrient uptake in intact intestinal tissue was used (Westergaard and Dietschy, 1974). The effects of short-term exposure to varying concentrations of both calcium channel blockers (CCBs) on short circuit current (Isc) were also determined.

A) Acute Experiments

In the acute experiments, two groups of 6 animals were maintained on chow (hereafter "low cholesterol diet" or "LCD") and two groups were fed the same pellets modified to contain 2.8% cholesterol (w/w) referred to as the "high cholesterol diet" or

"HCD". The HCD was prepared by adding cholesterol (5-Cholesten-3β-ol; Sigma Chemical Co., St. Louis, MO) to the low cholesterol pellets. Briefly, 4 kg allotments were thoroughly mixed with 100 g of cholesterol dissolved in 500 g of ethyl ether (reagent grade; Fisher Scientific Ltd., Fair Lawn, NJ). The prepared food mixture was then spread out on trays and placed in a fume hood to dry for 48 hours. To validate this method the cholesterol content of the prepared pellets was determined using a modified Folch's lipid extraction procedure (Bowyer and King, 1977; Morin, 1976) and a commercial enzymatic colorimetric test for cholesterol determination (Boehringer Mannheim, West Germany). The cholesterol content was determined to be 2.8%±0.2 (mean±SEM).

Animals used in the Isc experiments were prepared in a similar manner with nine rabbits in the LCD group and eight in the HCD group.

B) Chronic Experiments

For the chronic drug experiments animals were divided into "no drug" groups including LCD (n=6 in 2 groups) and HCD (n=6 in 2 groups) and "drug administered" groups including LCD plus N (n=6), LCD plus V (n=6), HCD plus N (n=6) and HCD plus V (n=6).

3.2.3) DRUG DOSES AND ADMINISTRATION

All feeding treatments were for a 3 week duration. Solubilized N and V (as described below) were provided orally by syringe at daily doses of 1 mg/kg body weight and 4 mg/kg, respectively. The dose of N was selected to duplicate the model of Senaratne et al (1991) and to avoid hemodynamic effects of the drug. A dose of 1 mg/kg N effectively reduces atherosclerosis and is well below the dose of 20 mg/kg day which was previously shown to decrease mean arterial pressure in cholesterol-fed New Zealand White rabbits (Fronek, 1988). The V dose of 4 mg/kg was selected to approximate clinical doses used in humans (240-360 mg/day on average for a 70 kg body weight adult) (McTavish and Sorkin,

1989) rather than the higher doses used in most reported studies (Rouleau et al, 1983; Blumlein et at, 1984). Furthermore, this dose of V is also not associated with hemodynamic effects (Blumlein et al, 1984).

Preliminary studies with the administration vehicle used in this study (i.e. introduction of aqueous solution by a syringe into the mouth) were shown to be without influence upon the effect of cholesterol feeding (unpublished observations, Kappagoda, 1989). Thus, a control vehicle was not used in the present study.

3.2.4) SERUM CHOLESTEROL AND TRIGLYCERIDE DETERMINATION

The serum cholesterol, triglyceride and glucose levels for all animals in the study were determined. Measurements were determined by the use of an automated system (Multistat III, Instrumentation Laboratories, Lexington, KY) which incorporated the methods of Allain et al (1974) and Pinter et al (1967).

3.3) IN VITRO UPTAKE TECHNIQUE

3.3.1) CHEMICALS

Unlabelled D-glucose, and L-glucose were greater than 99% pure as supplied by Sigma Chemical Co. (St. Louis, MO). D-galactose was obtained from Fisher Scientific Ltd. (Fair Lawn, NJ). The compound used to measure the adherent mucosal fluid volume, [^{3}H]inulin (molecular weight approximately 5000) was obtained from Amersham Canada Ltd. (Oakville, ON). The suppliers of the [^{14}C]-labelled compounds include D-glucose and galactose from Amersham Canada Ltd. (Oakville, ON) and L-glucose from ICN Biomedical Inc. (Montreal, PQ). All other compounds used were of reagent grade as supplied by Fisher Scientific Ltd. (Fair Lawn, NJ).

Nisoldipine (N), in powdered form, was provided as a gift from Miles Pharmaceutical Ltd. (New Haven, CT). The drug was solubilized in 3.5 mL of 95% ethanol

(<1.5% of final administration volume) and distilled deionized water for oral administration. Verapamil-HCl (V) was obtained from Sigma Chemical Co. (St. Louis, MO) and was solubilized in distilled deionized water. The drugs were concentrated in the solution so that the average quantity of fluid provided daily to achieve the required dose was 0.35 mL. The mean quantity of ethanol being received each day in the N administered rabbits was 5.4 μL. This is well below the amount of chronic ethanol intake (approximately 26 mL/day for 6-7 weeks) associated with altered uptake of lipids and glucose in rabbit jejunum (Thomson, 1984)

3.3.2) PREPARATION OF INCUBATION SOLUTIONS

Test solutions containing either D-glucose, galactose or L-glucose were prepared as described previously (Thomson and Dietschy, 1980). Briefly, the solutions were prepared by adding appropriate amounts of both unlabelled and [^{14}C]-labelled substrates to 150 mL of Krebs bicarbonate buffer. Appropriate amounts of [^{3}H]inulin were added and all solutions were stirred with a magnetic bar for 3 hours. The test solutions for D-glucose and galactose included concentrations of 2, 4, 8, 16, 32 and 64 mM. L-glucose concentrations included 4, 8 and 16 mM. For the acute studies test solutions were prepared as described above with the addition of N or V in concentrations ranging from 10^{-8}M to 10^{-4}M.

All preparation and experimentation with N was conducted in a darkened room to prevent photodegradation of the drug.

3.3.3) TISSUE PREPARATION

The rabbits were anesthetized with pentobarbitone sodium (25 mg/kg) and the jejunal segment was quickly removed and rinsed with 150 mL of ice cold saline. The segment was opened along the mesenteric border, rinsed gently with 50 mL saline and was cut into several smaller sections of approximately 1.5 cm length. These sections were then mounted

as flat sheets in incubation chambers clamped between two plastic plates to allow exposure of the mucosal surface through an aperture of exactly 1.4 cm in diameter.

The mounted chambers were placed in beakers containing 150 mL of oxygenated Krebs-bicarbonate buffer at 37°C for a preincubation period of 30 minutes. All solutions were mixed at identical rates with circular magnetic bars to reduce the resistance of the intestinal unstirred water layer. This allowed for the better assessment of the transport properties of the brush border membrane (Westergaard and Dietschy, 1974). The stirring rate in this study was adjusted precisely to 600 revolutions per minute using a strobe light.

For acute exposure experiments half of the tissues were preincubated in Krebs-bicarbonate containing N or V ranging from 10^{-8}M to 10^{-4}M for 30 minutes, while the other half were preincubated in the drug free solution for the same time period.

3.3.4) DETERMINATION OF RATES OF UPTAKE

After the 30 minute preincubation period the transport chambers were transferred to beakers containing the radiolabelled test solutions for a strictly timed incubation period of 6 minutes. The experiment was then terminated by removing the chamber and quickly rinsing the mounted tissue in ice cold saline. The tissue segments were cut out from the transport chamber using a circular steel punch, placed on glass slides and dried overnight at 55°C in a drying oven. After determining the dry intestinal weight the tissue pieces were transferred to scintillation vials and saponified in 0.4 N sodium hydroxide. Scintillation fluid was added and radioactivity was determined by means of an external standardization technique to correct for variable quenching of the two isotopes. The rate of uptake was calculated after correcting the total tissue [^{14}C]-radioactivity for the mass of probe molecule present in the adherent mucosal fluid.

The rate of lipid uptake was expressed as the nmol of probe molecule taken up into the mucosa per 100 mg dry weight of mucosa (nmol/100 mg mucosa·min^{-1}). The weight

of the mucosa as well as the ratio of mucosa:submucosa was determined by scraping the mucosal layer from the underlying layer with a glass slide. Uptake rates were also calculated as nmol of probe taken up per minute per unit serosal surface area.

Rates of uptake were plotted as a function of substrate concentration for D-glucose and galactose. Active transport was determined by subtracting the passive contribution (L-glucose uptake) at each concentration. The relationship between hexose concentration and uptake was curvilinear and a plateau was achieved after removing the passive component. Maximal transport capacity and apparent affinity constants for D-glucose and galactose were estimated using the statistical software Systat (Evanston, IL) and weighted nonlinear regression techniques as previously described (Meddings et al, 1989).

The values obtained for the different groups of animals are reported as the mean±SEM of the results from 6-12 animals in each group.

3.4) SHORT CIRCUIT CURRENT STUDIES

3.4.1) CHEMICALS

Theophylline was purchased from Eastman Kodak Co. (Rochester, New York, NY). D-glucose, fructose and all other agents were obtained from Fisher Scientific Ltd. (Fair Lawn, NJ). The Ringers-HCO3 buffer used in the study included NaCl 114 mM, KCl 5 mM, Na2HPO4 1.65 mM, NaH2PO4 0.3 mM, MgCl2 1.1 mM, CaCl2 1.25 mM, NaHCO3 25 mM.

3.4.2) STUDY DESIGN

Using a protocol similar to that of Homaidan et al (1989) proximal jejunum was used to study concentration-dependent effects of calcium channel blockers (CCBs) on basal and stimulated short circuit current (Isc). Animals were anesthetized (as per the in vitro uptake method) and a 10 cm segment of proximal jejunum was quickly removed, rinsed with Ringers-HCO3 solution and stripped of serosa and muscularis propria. Four segments of the

prepared tissue from the same animal were immediately mounted in modified lucite Ussing chambers having an aperture of 0.786 cm^2. Oxygenated, 37°C Ringers-HCO_3 solution with 20 mM fructose bathed mucosal and serosal surfaces and all tissues were stabilized for 15 minutes before the initiation of treatments.

All CCB agents were added to the serosal bathing fluids. In two of the chambers increasing concentrations (10^{-7} to 10^{-5} x 3.16 M) of either nisoldipine (N) or verapamil (V) were added. In a third chamber the same concentrations of N were added followed by sequential addition of the same concentrations of V. In the fourth chamber the tissue served as a control.

To test effects of CCBs on stimulated Isc, 20 mM glucose was added to both sides of each chamber followed by addition of 1 mM theophylline on each side.

A dual voltage clamp instrument (DVC-1000) (World Precision Instruments Inc., New Haven, CT) was used to measure transmural potential difference (PD) as well as Isc. An automatic voltage clamp provided continuous current (i.e. Isc) to nullify the spontaneous PD except for the 10 second timed intervals when the PD was recorded. Isc and PD were determined every 2 minutes. Fluid resistance compensation (to correct for fluid-mediated voltage decreases between voltage electrodes and the membrane) was adjusted as per the instructions for the DVC-1000.

3.4.3) MORPHOLOGY

The morphology of the jejunal tissue was assessed as previously described (Ecknauer, 1982; Keelan et al, 1985a, 1985b). Morphological measurements were done on 1 cm sections of tissue fixed in Bouin's solution, dehydrated and embedded in paraffin. Sections were cut at a thickness of 5 μm for light microscopy, mounted on glass slides and stained with hematoxylin and eosin using standard techniques. A Scopion projecting microscope was used to project the tissue sections at 1000-fold magnification. Measurements

of villus height, villus width at 1/2 height, villus bottom width and crypt depth were obtained from vertical tissue sections. Measurement of villus thickness (a second dimension of villus width) was obtained from transverse tissue sections.

The villus surface area (VSA, μm^2/villus) was calculated according to the following formula:

$$VSA = (2xMxH)+(2xM-A)xD+(2xDx(((A-M^2)+(H)^2))^{0.5}x1000$$

where H = villus height, M = villus width at 1/2 height, A = villus bottom width , D= villus thickness at 1/2 height. To estimate villus density, the number of villi per mm were measured in longitudinal and horizontal cross-sections, and were then multiplied together to obtain the number of villi per mm^2 serosa. At least 10 villi were assessed per section. The mucosal surface area (MSA, mm^2/mm^2 serosa) was calculated from the formula:

MSA = number of villi/mm^2 serosa x VSA (mm^2/villus).

3.4.4) STATISTICAL ANALYSIS

The statistical significance of the difference between means was determined using a two-way analysis of variance procedure followed by the Student-Neumann Kuels multiple range test and unpaired t-tests to determine differences between individual treatment means. To determine if the hexose transport curves complied with either a one or two transport system model the statistical software Systat (Evanston, IL) and a weighted nonlinear regression technique was employed as described previously (Meddings et al, 1989).

3.5) RESULTS

3.5.1) ANIMAL CHARACTERISTICS

The oral administration of nisoldipine (N) and verapamil (V) was well tolerated, and there were no deaths or significant adverse events during the 3 week course of the study. All rabbits consumed the same amount of food, with the exception of the higher food intake

in animals fed the low cholesterol diet (LCD) plus N as compared with LCD, yet body weight gain was equal among all groups (Tables 3-1 - 3-3).

As expected, serum cholesterol and triglyceride concentrations were higher in high cholesterol diet (HCD) as compared with LCD. Adding V to LCD or HCD did not significantly affect these lipid levels. However, rabbits fed HCD plus N (Table 3-2) had lower serum cholesterol levels (880.6±93.6 mg/dL in HCD versus 587.5±22.6 mg/dL in HCD plus N). Although serum triglyceride levels were lower in HCD plus V versus HCD (Table 3-3), this difference was not statistically significant. Serum glucose levels were similar in LCD or HCD with or without N or V (Tables 3-2 and 3-3).

3.5.2) INTESTINAL MORPHOLOGY

Adding N or V to HCD lowered the dry weight of the jejunum (HCD plus N or HCD plus V versus HCD), but adding calcium channel blockers (CCBs) to LCD did not affect the weight of the jejunum (Table 3-4). Because of these differences in jejunal weight, and the lower percentage of the intestinal wall comprised of mucosa in HCD plus V, rates of uptake were expressed on the basis of the dry weight of the mucosa rather than the dry weight of the entire wall of the jejunum.

The villus height was significantly increased in the HCD plus N and HCD plus V groups as compared to the HCD animals not given CCBs, but because of adjustments in the dimensions of the width and thickness of the villus, the mucosal surface area was not altered among any of the animal groups.

3.5.3) HEXOSE UPTAKE

A) Acute Studies

The jejunal uptake of 20 mM glucose was unaffected by 6 or 36 minute exposure to increasing concentrations of N or V (Tables 3-5 and 3-6). The animals fed HCD exhibited

consistently reduced glucose uptakes compared to LCD. This effect was observed regardless of the concentration of drug present in the test solution.

B) Chronic Studies

The maximal transport capacities (Jmax) of both glucose and galactose were increased when N was fed with LCD and HCD (Table 3-7). The value of the apparent affinity constant (Km*) for glucose was unchanged between groups, but the Km* for galactose was higher in LCD plus N than in LCD, and was higher in HCD plus N than in HCD.

The feeding of V had effects that differed from N feeding, and the effects of V were also variable between LCD and HCD (Table 3-8). For example, the Jmax for glucose was lower in LCD plus V than in LCD, yet was higher in HCD plus V than in HCD. The effects of V on galactose Jmax were the opposite, with Jmax higher for LCD plus V than LCD, and lower in HCD plus V than in HCD. The Km* for both glucose and galactose were similarly affected by V, but the cholesterol content influenced the direction of these changes: the Km* for both hexoses decreased in LCD plus V animals compared to LCD, but increased in HCD plus V animals compared to HCD.

The apparent passive permeability of glucose and presumably of galactose was assessed from the uptake of L-glucose (Tables 3-7 and 3-8). L-glucose uptake was lower in LCD plus N but higher in LCD plus V than in their respective control LCD groups.

3.5.4) SHORT CIRCUIT CURRENT STUDIES

Increasing concentrations of CCBs added to the serosal bathing solution did not affect jejunal basal short circuit current (Isc) in either LCD or HCD fed animals (Figures 1 and 2). However, CCBs reduced D-glucose- and theophylline-stimulated Isc in the LCD group. Stimulated Isc was determined by addition of 20 mM D-glucose followed by 1 mM theophylline to both the mucosal and serosal solutions (the latter containing the previously added CCBs). The change in Isc (ΔIsc) under each condition was determined by comparing

the maximally stimulated Isc value to the baseline Isc just prior to addition of the stimulant (Figures 1 and 2). The addition of 20 mM D-glucose increased Isc in all LCD tissues, although the magnitude of the stimulation was significantly less in the tissues exposed to CCBs. In contrast, in the HCD group D-glucose stimulated ΔIsc was significantly less in control tissues (i.e. not exposed to CCBs) than the LCD group. Exposure of jejunal tissue from HCD animals to CCBs did not further reduce D-glucose stimulated changes in Isc (Figure 2). ΔIsc was similar in each group and did not change in the presence of CCBs.

Following stabilization of the D-glucose-stimulated Isc, 1 mM theophylline was added to both mucosal and serosal bathing solutions. A statistically significant increase in Isc was observed for all tissues, regardless of whether the CCBs were present or whether the animals were fed LCD or HCD. However, as in the case of glucose, ΔIsc was significantly lower in LCD, but not HCD tissues, exposed to CCBs.

Addition of the CCBs, glucose or theophylline had no significant effect on jejunal conductance (data not shown). Unexpectedly, however, the conductance of the LCD control tissue increased significantly (34%) during the first 30-40 minutes of the experiment and generally stabilized at a higher value than the other tissues. During the time of the rising conductance, the control LCD tissue was not exposed to any treatments, only the circulating oxygenated Ringers bicarbonate solution at 37°C.

3.6) DISCUSSION

The presence of two or more carriers for hexose transport has been suggested in previous studies (Thomson et al, 1987; Malo, 1990). Chronic oral administration of verapamil (V) had differential effects on glucose and galactose uptake, thus supporting the possibility of multiple intestinal hexose carriers. The results of this study also suggest the possibility of multiple calcium channels in the jejunum. For example, the effects of V on

both active and passive components of hexose uptake were qualitatively different from nisoldipine (N), a different class of calcium channel blocker. Furthermore, the effect of V on active hexose transport is significantly altered by the presence of high cholesterol levels in the diet whereas N effects are not different between the low and high cholesterol diet (LCD and HCD).

It is unlikely that the altered transport rates are due to direct effects of N or V in the intestinal lumen, as acute exposure of intact tissue to these drugs did not affect in vitro uptake of glucose (Tables 3-5 and 3-6). Thus, it is likely that the altered transport parameters are due to an adaptive response to the chronic administration of these drugs. It is possible that these adaptive phenomena are stimulated by chronically altered cellular influx of calcium.

The magnitude of calcium entry blockade, and therefore the effectiveness of various classes of calcium channel blockers (CCBs), may be variable in different tissues (Katz, 1986; Homaidan et al, 1989; Hof et al, 1990). It has been suggested that V-like drugs may exert quantitatively greater effects on short circuit current (Isc) and therefore on electrolyte fluxes in rabbit ileal tissue. The effects of V on the maximal transport rate (Jmax), apparent affinity constant (Km^*) and apparent passive permeability coefficient (Pd^*) differed from those of N (Tables 3-7 and 3-8). Whether this is a qualitative difference between different classes of CCBs, or is attributable to different magnitudes of calcium blockade, is uncertain. Alternatively, in nonintestinal tissues CCBs may have sites of action apart from the voltage-sensitive channels (Weinstein and Heider, 1987) including blockade of sodium channels (Yatani and Brown, 1985) and stimulation of the sodium ion pump (Pan and Janis, 1984).

N effects on the kinetic parameters of both glucose and galactose transport appeared to be independent of the dietary content of cholesterol. However, there was a

clear effect of cholesterol feeding on V-induced alterations in hexose uptake. A similar trend was noted in a study of lipid uptake in which the combination of HCD and V had opposite effects on cholesterol and fatty acid uptake as compared to the LCD groups (unpublished observations, Hyson et al, 1991). It is possible that cholesterol feeding alters the lipid environment of the calcium channel(s) to expose binding sites that would otherwise be unavailable for interaction with the CCBs. This phenomenon has been suggested in rabbit aorta, where it was observed that V and diltiazem were able to antagonize the effect of norepinephrine-stimulated calcium transport in cholesterol-enriched but not in control samples (Bialecki and Tulenko, 1989). Since different classes of CCBs appear to bind to different sites on calcium channel proteins (Glossman et al, 1985), it may be speculated that cholesterol feeding alters the lipid composition and fluidity of the brush border membrane and thereby affects the binding site for V differently from that of N (Brasitus, 1987).

The passive component of intestinal glucose uptake was also differentially affected by N and V, but only in animals fed LCD. In this case, the high cholesterol content of the diet appears to have exerted a "protective" effect against the CCB-induced changes, as there were no alterations in Pd^{*} in either HCD plus N or HCD plus V compared to their respective HCD controls. It is well documented that calcium is an important factor contributing to the integrity of tight junctional complexes or strands (Donowitz and Madara, 1982; Madara and Marcial, 1984). Recently, it has been suggested that intracellular cAMP or calcium activation signals may influence the tight junctions in some epithelial tissues, possibly via direct manipulations through cytoskeletal interactions. The latter may be mediated by specific proteins associated with the tight junctions (Madara, 1989). It is therefore reasonable to speculate that the chronic administration of CCBs might alter the tight junctions of the intestine and that cholesterol-feeding might prevent these CCB-associated alterations.

Alterations to both the passive and active transport components contribute to the net effect of CCBs on hexose uptake. Passive glucose uptake may exceed rates of active transport at luminal concentrations in the physiological range after a meal (Pappenheimer, 1990). In the present study, feeding LCD plus V reduced Jmax by 17.6% but increased Pd^* by 29.4% versus LCD. If passive flux is 50% of the total glucose uptake after a meal (Pappenheimer and Reisse, 1987), it may be speculated that V feeding results in a slight (6%) increase in net glucose uptake. N feeding enhanced Jmax by 29.5% and reduced Pd^* by 12.2%; based on the above assumption, the net effect might be an 8.8% increase in glucose uptake. In HCD, Pd^* was not affected but Jmax increased with N or V feeding. Thus, it is possible that CCBs differentially affect individual kinetic parameters, but their effects on net glucose uptake may be directionally similar.

Several intestinal adaptive mechanisms to various stimuli have been described (Thomson et al, 1989, 1990). For example, effects on the unstirred water layer (UWL) adjacent to the cells may account for altered nutrient transport in response to aging, diabetes and dietary manipulation (Thomson, 1984). UWL resistance was not measured in this study, but a change in UWL with N or V would be expected to affect the value of Km^* and Pd^* in a similar direction, and this did not occur (Tables 3-7 and 3-8). Furthermore, changes in UWL would not influence the value of Jmax, which was observed in this study. Thus, it is unlikely that alterations in UWL resistance played a major role in the mechanism of the effects of N or V on hexose uptake in LCD or HCD.

The results of this study cannot be explained on the basis of variations in the animals' body weight gain or food intake (Tables 3-1 - 3-3). The villus height in both the HCD plus N and the HCD plus V animals was significantly increased, but there was no difference in the total mucosal surface area (Table 3-4). This was likely due to the reduced density of the villi (number of villi/mm^2 serosa) in the HCD plus N and HCD plus V groups.

It is clear that the inclusion of three dimensional measurements such as villus thickness and density provide a more reliable estimate of the alterations in mucosal surface area in this study. As there were no differences in the mean mucosal surface areas among the animal groups (Table 3-4), the effects of N and V on glucose uptake in LCD or HCD could not be explained by changes in the static morphology of the intestine.

It should be noted however that a lack of correlation between mucosal surface area and nutrient transport function has been reported in other studies (Keelan et al, 1985a, 1985c). Recent studies have suggested that a specialized portion of the villus, notably the upper one-third, is used for the uptake of glucose and amino acids (Chang et al, 1987; Maenz and Cheeseman, 1986; Cheeseman, 1986). Changes in total villus surface area may not necessarily be associated with changes in the functional surface area used for nutrient uptake. Thus, the lack of a change in villus surface area in an experimental setting does not necessarily signify the lack of change in functional villus surface area.

Static measurements of mucosal surface area do not indicate which portion of the villus and which functional component may be affected, nor do they provide information regarding the dynamic aspects affecting morphology and transport function including, rates of cell proliferation, differentiation, migration and turnover. Alterations in any of these parameters could potentially affect the functional surface area involved in nutrient uptake. There is increasing evidence for the role of polyamines (putrescine, spermine and spermidine) in the intestinal adaptive process. It is recognized the increased polyamine synthesis is an early event in cell growth, replication and differentiation (Pegg and McCann, 1982). In the intestine ornithine decarboxylase (ODC) levels appear to be higher in villus than crypt cells, implying a possible polyamine involvement in mature, as well as differentiating cells (Baylin et al, 1978; Sepulveda et al, 1982). It has been demonstrated in vitro that calcium may play a role in inducing ODC, the rate-regulating synthetic enzyme for

polyamines (Langdon, 1984; van Leeuwen, 1988). Furthermore, V dose-dependently reduced basal and stimulated ODC levels in cultured osteogenic sarcoma cells (UMR 106-01) (van Leeuwen, 1988). These authors suggested a role for calcium in basal ODC regulation, and a calcium, cAMP dependent mediation of stimulated ODC activity. It is unknown what the effects of chronic CCB feeding on intestinal ODC activity might be. It is interesting to speculate that feeding chronic CCBs might reduce intestinal ODC levels and influence enterocyte proliferation, migration, differentiation and/or turnover rates. For example, reduced enterocyte turnover and migration rates might allow the enterocytes remaining on the villus to mature and acquire greater transport potential. A more mature population of cells or altered distribution of transporting enterocytes would be expected to be associated with an enhanced Jmax. Alterations in the distribution of transporting cells and "recruitment" of carriers to the lower portion of the villus have been suggested as possible adaptive mechanisms to experimental stimuli such as diabetes or radiation (Chang et al, 1987; Thomson et al, 1989). However, due to the variable nature of the observed hexose uptake responses the effects of CCB's on functional surface area are likely more complex. For example, while villus height is significantly increased in HCD plus N or HCD plus V groups, Jmax increases for both hexoses in HCD plus N group but only glucose Jmax increases in HCD plus V animals; the Jmax for galactose is in fact, lowered in HCD plus V. Furthermore, in the case of unaltered villus height (i.e. LCD plus N and LCD plus V groups) glucose and galactose Jmax are increased in the LCD plus N group but only galactose Jmax is increased in LCD plus V animals; the Jmax for glucose is lower in LCD plus V compared to LCD controls. Thus, it is difficult to propose a simple model to explain these findings.

In vitro CCBs have been associated with the de novo synthesis of LDL receptors, suggesting their potential for synthetic activity of membrane-bound proteins (Paoletti et al, 1988). Whether CCBs could act in a similar way to promote the synthesis of one or more

membrane-bound hexose transporters is an intriguing possibility. The induction of intestinal glucose transporters has been proposed as a mechanism for the enhanced Jmax observed in response to high carbohydrate diets fed to mice (Diamond and Karasov, 1984). If CCBs stimulate synthesis of glucose carriers this might account for the lack of effect of the CCBs on glucose uptake in the acute studies where tissue exposure to the agents was limited to 6 and 36 minutes. However, once again, the reasons for the decreased Jmax for glucose and galactose in the LCD plus V and HCD plus V group, respectively, are unclear.

In addition to Jmax, the Km* of the carrier for the hexoses was affected by the chronic feeding of CCBs. Thus, it is apparent that the CCBs mediate an alteration in the property of the existing carrier(s), as well as a possible change in their number. It is well known that lipid composition determines the physicochemical properties of the membrane and may have significant effects on the function of membrane-bound proteins (Yeagle, 1989; Clandinin et al, 1985). It has been reported that membrane lipid composition and fluidity have significant effects upon the kinetic parameters of glucose transport in rabbit and rat intestinal brush border membrane vesicles (Brasitus and Schacter, 1982; Meddings et al, 1990). It is not known if CCBs influence the composition of the intestinal membrane or the lipid-metabolizing enzymes in the enterocyte. A calcium, calmodulin-dependent kinase has been suggested to play a role in the short-term regulation of HMG-CoA reductase, the rate limiting enzyme for cholesterol synthesis (Beg et al, 1987a, 1987b). Calcium may play a direct role in modulating membrane lipids by binding to anionic sites of the membrane bilayer. However, in rat brush border membrane vesicles intravesicular calcium decreased the fluidity by alterations in membrane sphingomyelin content by unknown, presumably indirect mechanisms (Dudeja et al, 1987). Calcium-mediated alterations in lipid composition have also been observed in hepatocytes (Livingstone and Schacter, 1980). Possible calcium involvement in acylation and deacylation enzymes in the enterocyte (Garg et al, 1988) has

not been studied. However, it might be anticipated that any calcium mediated-step would be qualitatively affected in a similar manner by both classes of CCBs. Thus, it would be expected that similar changes in the Km"s would be observed for both V and N; this was not the case in the present study.

Alterations in the sodium electrochemical gradients across the cell would be expected to affect the movement of sodium-dependent substrates such as glucose and galactose. CCBs have been shown to alter sodium permeability in Isc studies of ileal tissue: V has a quantitatively greater effect than the other classes of CCBs in dose-dependent reduction of basal Isc (Homaidan et al, 1989). The Isc changes are suggested to reflect the effect of calcium channel blockade on the flux of anions and cations from mucosa to serosa and vice versa. In the presence of CCBs equally enhanced mucosal to serosal movement of sodium and chloride exceeds serosal to mucosal movement of these ions. Residual fluxes (likely bicarbonate ions) and Isc are decreased. However, glucose-dependent sodium fluxes are unaffected after short-term exposure to CCBs in rabbit ileal tissue, implying no effect of CCBs on sodium-dependent glucose transport (Hubel and Callanan, 1980; Donowitz and Asarkof, 1982). The results of our acute uptake studies support this observation (Tables 3-5 and 6). However, in our chronically fed animals it is apparent that glucose transport is significantly altered by the CCBs (Tables 3-7 and 3-8). Thus, the effects of short-term exposure to CCBs differ from those of chronic feeding of the drugs, and the chronic effects are not explained by the presence of the drug in the bulk phase or in the enterocyte.

In nonintestinal cells, reductions in intracellular calcium induce alterations in sodium permeability; these effects are associated with enhanced activity of the sodium pump (Na^+/K^+-ATPase) (Hagane et al, 1989; Knudsen and Johansen, 1989). Whether calcium is involved in the physiological regulation of the sodium pump and whether chronically fed CCBs exert an influence on Na^+/K^+-ATPase in the intestinal basolateral membrane is

unknown. There appear to be at least two isoforms of this ATPase in the intestine (Fedorak, 1990) which could be differentially affected by cholesterol feeding and could be influenced differently by various classes of CCBs. However, the variable effect of V on the uptake of glucose and galactose suggests that a general effect on sodium gradients is unlikely the sole mechanism mediating the changes in nutrient transport.

Acute and chronic effects of CCBs may differ in jejunal tissue compared to ileum. We were unable to demonstrate in the jejunum the reported (Homaidan et al, 1989) dose-dependent reduction in basal Isc by CCB exposure to rabbit ileum. This lack of effect could be related to differing electrolyte transport mechanisms between jejunum and ileum. Specifically, in rabbit jejunum there does not appear to be either a Na^+/Cl^- symport or a $Cl^-/0H^-$ antiport (Gunther and Wright, 1983). Since ion flux studies were not done in our study, it is not possible to comment on the direction or specific ions contributing to the Isc.

Glucose-stimulated Isc responses were suppressed by the presence of V, N and N plus V in the serosal bathing solution. This result was observed in LCD but not in HCD. However, the control HCD Isc response to glucose was lower than the LCD control values. Thus, since the glucose response was already suppressed in the HCD versus LCD, this may have accounted for the lack of further depression by CCBs. As discussed earlier, the feeding of cholesterol might alter the tissue properties in a manner that influences the interaction between CCBs and the calcium channels. Furthermore, as discussed earlier, the fluidity and physicochemical properties of the membrane may be altered by HCD, resulting in altered transport function. It is also possible that cholesterol alters the properties of the tight junctions and thus the general permeability of the tissue. For example, the ionic conductance in the LCD control tissue was greater than the conductance in the HCD group. It is unlikely that these results were due to toxic effects or tissue death as theophylline significantly increased Isc in all tissues.

The reduced Isc response to glucose in the CCB exposed LCD tissues, presumably reflects diminished sodium-dependent mucosal to serosal movement of the hexose. These results were unexpected. Based on the enhanced mucosal to serosal flux of sodium in the presence of these drugs (Hubel and Callanan, 1980; Donowitz and Asarkof, 1982), it might be anticipated that increased glucose movement in the same direction would occur. Furthermore, the results of our acute exposure study did not show any effect of increasing doses of mucosal N and V on in vitro glucose uptake. Based on these results in the acute study, it is unlikely that a mucosal effect (i.e. modification of the glucose carrier(s) in the brush border membrane) is occurring after short-term exposure to CCBs. However, it might be speculated that CCBs reduced the activity of calcium-dependent potassium channels (CDPCs) proposed to exist in enterocytes (Brown and Sepulveda, 1985; Montero et al, 1990). Increased potassium permeability at the basolateral membrane may be coordinated with enhanced sodium entry across the apical membrane and it has been suggested that CDPCs may mediate this linkage. If potassium permeability plays a role in providing the driving force for sodium-coupled transport across the cell it is possible that CCBs could influence the movement of glucose by affecting the activity of the CDPCs.

The implied CCB suppression of glucose transport in the Isc study differs from the results of our acute and chronic exposure in vitro uptake studies. The time-course of drug exposure may be a factor. In the acute exposure nutrient uptake studies the tissues were exposed to CCBs for 6 and 36 minutes, whereas in the Isc studies, CCBs were present in the medium for 72 minutes before the glucose-stimulated measurements were determined. Furthermore, as indicated above, the mucosal versus serosal exposure to CCBs may have significance. Additional Isc studies with mucosal addition of the CCBs need to be conducted to determine if the site of CCB exposure has an influence in the jejunum.

It is not unexpected that the functional effects of the chronic study would differ from the acute exposure studies. In acute experiments of drug exposure, the drug is directly exposed to either the mucosal or serosal surface of the tissue being studied. However, in a chronic administration study of CCBs, several factors need to be considered. In this study, the CCBs are administered orally and thus present to the luminal side of the enterocyte. Thus, the absorption of the agents may be variable and influenced by the luminal contents. In view of the lipophilic nature of these agents it is likely that their absorption will be enhanced in the presence of a high lipid diet. We attempted to avoid a possible differential effect in drug absorption between HCD and LCD by providing the drug at noon, when food intake by this species is generally low. However, variable absorption between drugs and between diets cannot be ruled out.

Another consideration with the luminal presentation of the drug is that calcium channels are present predominantly in the basolateral membrane of the ileum (Donowitz, 1983). If this is the case in the jejunum, then the pharmacological agents must cross the enterocyte before exerting their blockade effect on the basolateral membrane. Although the presence of luminal calcium channels has been implied in jejunum and colon (Markowitz et al, 1985; Donowitz et al, 1985) this is uncertain. Thus, it is possible that the observed effects are not exclusively due to the calcium channel blocking effects but also luminal and other unidentified processes occurring during transport across the intestine. This does not make the results of this study less significant, as the majority of these drugs are taken orally. In terms of hemodynamics, the administration of oral versus injected CCBs exert qualitatively similar effects (McTavish and Sorkin, 1989). In order to further delineate possible mechanisms of the results obtained in these chronic feeding studies, the use of intraperitoneal injections of these agents would be of interest.

It is unknown what effects CCBs have on mesenteric blood flow, and what implications this might have on in vivo nutrient uptake. In the present study drug doses were selected to avoid hemodynamic effects; thus it is unlikely that there would be a significant alteration in blood flow (Hof and Hof, 1989).

The physiological significance of the findings in this study remain to be determined. Oral V has been reported to reduce glucose tolerance to a glucose load in normal but not in diabetic volunteers (Ferlito et al, 1982). The mechanism may be related to altered cell permeability to glucose. The effects on intestinal uptake were not determined. Other studies have reported negative effects of CCBs on glucose tolerance (McTavish and Sorkin, 1989). In this study, the animals fed the CCBs had normal serum glucose levels in spite of altered capacities for intestinal glucose transport.

TABLE 3-1: Characteristics of animals used in the study of acute drug exposure.

	LCD	HCD
WEIGHT GAIN (kg/3 weeks)	0.71±0.06	0.72±0.07
TOTAL FOOD CONSUMED (g/3 weeks)	2326	2468
CHOLESTEROL INTAKE (g/3 weeks)	n/a	61.71
SERUM PARAMETERS (mg/dL)		
Cholesterol (0)	101.5±8.9	96.6±6.1
Cholesterol (3)	87.9±5.8	1675.7±166.9[a]
Triglyceride (0)	131.8±12.1	107.4±12.4
Triglyceride (3)	108.5±9.8	211.4±39.3[a]
Glucose (0)	166.9±17.8	135.4±5.8
Glucose (3)	133.3±4.7	120.4±2.3

Values are mean±SE; LCD = low cholesterol diet; HCD = high cholesterol diet;

(0) = value at initiation of study; (3) = value after 3 week feeding; n/a = not applicable

[a] $p<0.05$ LCD versus HCD

TABLE 3-2: Characteristics of nisoldipine (N)-administered and control animals.

	LCD	LCD+N	HCD	HCD+N
WEIGHT GAIN (kg/3 weeks)	0.64±0.03	0.63±0.05	0.64±0.05	0.57±0.05
TOTAL FOOD CONSUMED (g/3 weeks)	1614±54	1917±119[a]	1977±104	1805±106
CHOLESTEROL INTAKE (g/3 weeks)	n/a	n/a	49.41±2.58	45.09±2.65
SERUM PARAMETERS (mg/dL)				
Cholesterol (0)	103.4±9.5	107.3±6.0	108.9±9.6	105.3±5.8
Cholesterol (3)	73.1±4.1	63.9±3.3	880.6±93.6[b]	587.5±22.6[b,c]
Triglyceride (0)	169.9±53.7	123.0±24.8	156.4±24.9	137.0±21.5
Triglyceride (3)	91.4±14.1	87.1±9.4	126.7±15.8	139.8±31.9
Glucose (0)	154.7±9.4	165.4±7.6	146.8±5.1	153.4±4.9
Glucose (3)	161.6±12.5	147.1±14.1	144.7±6.1	139.6±8.1

Values are mean±SE; LCD = low cholesterol diet; LCD+N = low cholesterol diet plus nisoldipine; HCD = high cholesterol diet; HCD+N = high cholesterol diet plus nisoldipine; (0) = value at initiation of study; (3) = value after 3 week feeding; n/a = not applicable

[a] $p<0.05$ LCD versus LCD+N
[b] $p<0.05$ HCD, HCD+N versus LCD, LCD+N
[c] $p<0.05$ HCD versus HCD+N

TABLE 3-3: Characteristics of verapamil (V)-administered and control animals.

	LCD	LCD+V	HCD	HCD+V
WEIGHT GAIN (kg/3 weeks)	0.72±0.04	0.64±0.06	0.65±0.09	0.60±0.08
TOTAL FOOD CONSUMED (g/3 weeks)	2421±153	1946±77	2287±71	2427±254
CHOLESTEROL INTAKE (g/3 weeks)	n/a	n/a	57.15±1.76	59.82±7.04
SERUM PARAMETERS (mg/dL)				
Cholesterol (0)	62.4±3.9	100.4±9.3	103.1±5.9	71.5±8.1
Cholesterol (3)	57.3±5.9	70.4±6.2	1010.6±295.2[a]	992.1±217.8[a]
Triglyceride (0)	91.9±18.1	121.2±27.8	132.1±27.3	95.1±15.1
Triglyceride (3)	73.4±16.1	56.2±15.5	156.6±44.9[a]	108.6±27.2[a]
Glucose (0)	147.0±11.2	139.5±5.4	213.3±37.8	191.8±29.1
Glucose (3)	154.3±8.9	145.8±10.3	156.2±5.4	155.8±16.7

Values are mean±SE; LCD = low cholesterol diet; LCD+V = low cholesterol diet plus verapamil; HCD = high cholesterol diet; HCD+V = high cholesterol diet plus verapamil; (0) = value at initiation of study; (3) = value after 3 week feeding; n/a = not applicable

[a] $p<0.05$ HCD, HCD+V versus LCD, LCD+V

TABLE 3-4: Jejunal characteristics and morphology of study animals.

	LCD	HCD	LCD+N	HCD+N	LCD+V	HCD+V
Dry weight mg/unit serosal surface area	35.7±2.3	39.2±1.5	31.6±1.2	31.7±1.8[b]	34.1±1.3	32.5±1.6[c]
% of intestinal wall comprised of mucosa	84.1±1.8	83.6±1.1	78.4±3.0	82.9±1.9	78.9±2.1	72.2±1.2[c]
Crypt depth (μm)	50±2.9	43±2.5	51±2.6	69±3.0[b]	44±2.7	44±2.1
Villus height (μm)	603±29	599±24	573±25	717±33[b]	680±42	752±50[c]
Villus width at half height (μm)	94±4	126±6	98±4	158±8	114±6	124±6
Villus bottom width (μm)	114±7	135±5	122±5	166±6	127±8	134±7
Villus thickness (μm)	431±28	210±12	327±30	390±50	340±25	267±11
Villus surface area (μm^2/villus)	666±32	428±18	511±23	846±45	651±37	622±47
No. of villi/mm^2 serosa	21.3±1.4	35.6±1.2	25.3±1.0[a]	15.7±0.6[b]	24.2±1.7	28.8±1.5[c]
Mucosal surface area (mm^2/mm^2 serosa)	14.0±1.0	15.2±0.9	13.0±0.9	13.2±0.8	15.81±1.5	17.8±1.5

Values are mean±SE; LCD = low cholesterol diet; LCD+N = low cholesterol diet plus nisoldipine; LCD+V = low cholesterol diet plus verapamil; HCD = high cholesterol diet; HCD+N = high cholesterol diet plus nisoldipine; HCD+V = high cholesterol diet plus verapamil

[a] ($p<0.05$) LCD versus LCD+N
[b] ($p<0.05$) HCD versus HCD+N
[c] ($p<0.05$) HCD versus HCD+V

TABLE 3-5: Effect of acute tissue exposure to nisoldipine (N) on jejunal glucose uptake.

Incubation (M) Concentration of Nisoldipine	6 Minute Exposure to N		36 Minute Exposure to N	
	LCD	HCD	LCD	HCD
No Drug	161.26±16.77	59.69±8.93	161.26±16.77	59.69±8.93[a]
10^{-8}	53.49±15.64	39.92±14.15	149.46±22.34	57.05±21.60[a]
10^{-7}	97.35±12.40	112.71±16.78	115.96±15.56	152.92±27.50[a]
10^{-6}	132.38±18.54	84.28±13.92	84.55±11.80	41.67±11.06[a]
10^{-5}	134.28±22.96	78.01±19.79	125.18±20.21	55.99±6.83[a]
10^{-4}	90.01±14.36	85.92±15.13	137.76±33.07	37.34±4.85[a]

Values are mean±SE; LCD = low cholesterol diet; HCD = high cholesterol diet.

The rate of uptake of 20 mM glucose had the units nmol/100 mg mucosa·min^{-1}.

[a] Low cholesterol versus high cholesterol diet ($p<0.05$)

TABLE 3-6: Effect of acute tissue exposure to verapamil (V) on jejunal glucose uptake.

Incubation (M) Concentration of Verapamil	6 Minute Exposure to V		36 Minute Exposure to V	
	LCD	HCD	LCD	HCD
No Drug	272.32±22.51	274.13±18.51	295.57±13.12	266.80±14.39
10^{-8}	290.67±23.83	281.71±18.37	280.00±18.19	244.63±12.48
10^{-7}	250.31±17.18	238.37±17.78	350.10±22.56	241.92±17.34
10^{-5}	338.36±22.50	289.28±19.53	214.28±16.84	184.35±13.18

Values are mean±SE; LCD = low cholesterol diet; HCD = high cholesterol diet

The rate of uptake of 20 mM glucose had the units nmol/100 mg mucosa·min^{-1}.

TABLE 3-7: The effect of nisoldipine (N) on the values of maximal transport rate, apparent Michaelis constant and apparent passive permeability coefficient for glucose uptake into the jejunum of rabbits fed a low or high cholesterol diet.

	LCD	LCD+N	HCD	HCD+N
GLUCOSE				
Jmax (nmol/100 mg mucosa·min^{-1})	261.1±60.1	338.0±97.6[a]	203.1±36.6	276.8±26.7[b]
Km* (mM)	5.4±3.2	6.5±5.3	3.4±2.4	3.8±1.2
Pd* (nmol/100 mg mucosa·min^{-1})	4.1±0.2	3.6±0.1[a]	3.7±0.2	3.5±0.2
GALACTOSE				
Jmax (nmol/100 mg mucosa·min^{-1})	511.4±60.2	810.7±170.7[a]	453.3±86.3	572.9±28.6[b]
Km* (mM)	15.2±13.2	27.5±9.2[a]	11.2±4.8	16.8±1.6[b]

Values are mean±SE; LCD = low cholesterol diet; LCD+N = low cholesterol diet plus nisoldipine; HCD = high cholesterol diet; HCD+N = high cholesterol diet plus nisoldipine; Jmax = maximal transport capacity; Km* = apparent affinity constant; Pd* = apparent passive permeability coefficient. The value of the apparent passive permeability coefficient for galactose is assumed to be the same as for glucose.

[a] $p<0.05$ LCD versus LCD+N
[b] $p<0.05$ HCD versus HCD+N

TABLE 3-8: The effect of verapamil (V) on the values of maximal transport rate, apparent Michaelis constant and apparent passive permeability coefficient for glucose uptake into the jejunum of rabbits fed a low or high cholesterol diet.

	LCD	LCD+V	HCD	HCD+V
GLUCOSE				
Jmax (nmol/100 mg mucosa·min^{-1})	415.2±21.9	342.1±42.2[a]	443.6±28.3	552.8±35.6[b]
Km* (mM)	7.8±1.2	1.8±1.1[a]	3.4±0.7	6.9±1.3[b]
Pd* (nmol/100 mg mucosa·min^{-1})	3.4±0.2	4.4±0.2[a]	4.4±0.3	3.7±0.2
GALACTOSE				
Jmax (nmol/100 mg mucosa·min^{-1})	568.2±152.8	843.1±88.2[a]	718.8±104.8	608.7±122.7[b]
Km* (mM)	9.8±6.1	6.3±1.9[a]	3.4±1.8	7.8±4.5[b]

Values are mean±SE; LCD = low cholesterol diet; LCD+V = low cholesterol diet plus verapamil; HCD = high cholesterol diet; HCD+V = high cholesterol diet plus verapamil Jmax = maximal transport capacity; Km* = apparent affinity constant; Pd* = apparent passive permeability coefficient. The value of the apparent passive permeability coefficient for galactose is assumed to be the same as for glucose.

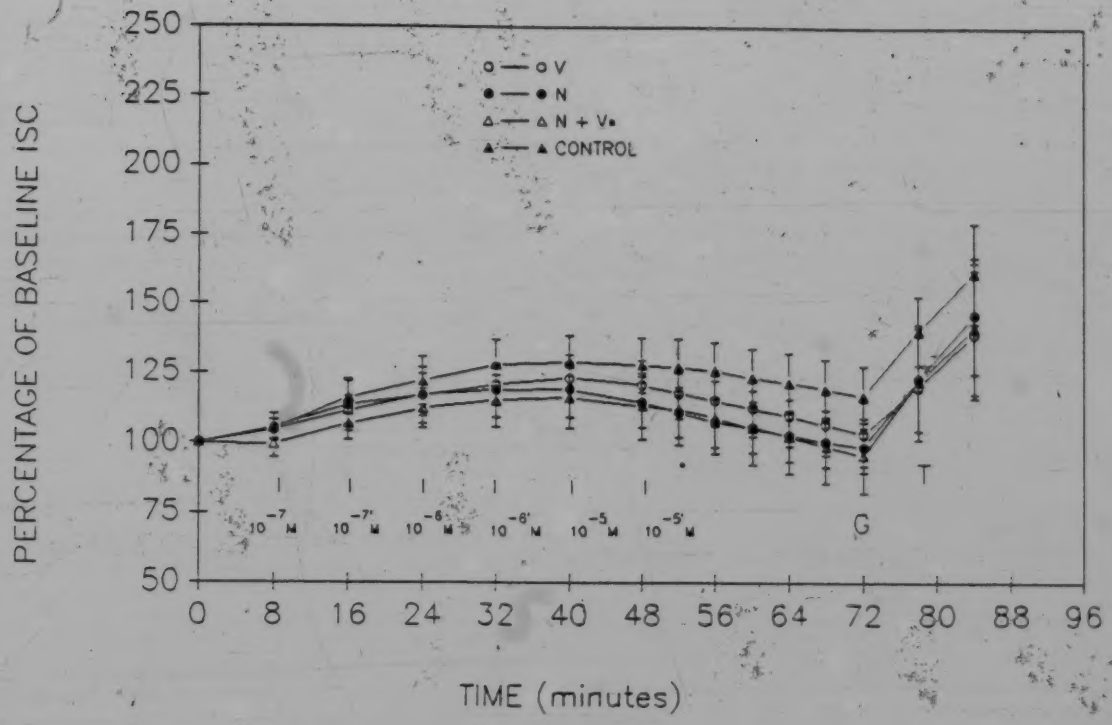
[a] $p<0.05$ LCD versus LCD+V
[b] $p<0.05$ HCD versus HCD+V

FIGURE 3-1: Effect of calcium channel blockers on short circuit current in LCD animals.

NOTE: Points = mean±SEM. All drugs added in concentrations ranging from 10^{-7}M to 10^{-5}Mx3.16 ("|"="x3.16"). V = verapamil; N = nisoldipine; N+V = nisoldipine followed by verapamil; Control = no added drug; G = addition of 20 mM D-glucose; T = addition of 1 mM theophylline.

[a] $p<0.05$ control versus V, N and N+V

FIGURE 3-2 Effect of calcium channel blockers on short circuit current in HCD animals.



NOTE: Points = mean±SEM. All drugs added in concentrations ranging from 10^{-7}M to 10^{-5}Mx3.16 ("|"="x3.16"). V = verapamil; N = nisoldipine; N+V = nisoldipine followed by verapamil; Control = no added drug; G = addition of 20 mM D-glucose; T = addition of 1 mM theophylline.

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CHAPTER 4

SUMMARIZING DISCUSSION

Chronic oral administration of nisoldipine (N) or verapamil (V) alters the in vitro jejunal uptake of lipids (cholesterol, stearic acid, oleic acid, linoleic acid) and hexoses (D-glucose, galactose and L-glucose). It is unlikely that these effects are due to direct activity of calcium channel blockers (CCBs) in the lumen since neither N nor V affected nutrient uptake when added directly to the incubation medium (Tables 2-5, 2-6, 3-5, 3-6). Thus, the effect of N or V is likely due to an adaptive process in the intestine occurring during the 3 weeks of feeding.

The magnitude of calcium (Ca) entry blockade and therefore the effectiveness of various classes of CCBs may be variable in different tissues (Katz, 1986; Homaidan et al, 1989). Differential effects between the two classes of CCBs on nutrient uptake were observed in this study. For example, the effects of V on the maximal transport rate (Jmax), apparent affinity constant (Km^*) and apparent passive permeability coefficient (Pd^*) differed from those of N (Tables 3-7 and 3-8). Qualitative differences in the effect of N or V on the uptake of fatty acids were also observed (Tables 2-7, 2-8). Thus, although the mechanisms of the effects of CCBs on nutrient uptake have not been established in this study, it appears the mechanisms may differ between these two classes of CCBs.

The effects of N on the kinetic parameters of both glucose and galactose transport as well as lipid uptake appeared to be independent of the dietary content of cholesterol. However, there was a dramatic effect of cholesterol feeding on V-induced alterations in nutrient uptake (Tables 2-7, 2-8, 3-7, 3-8). It is possible that cholesterol feeding alters the lipid environment of the Ca channel(s) to expose binding sites that would otherwise be

unavailable for interaction with the CCBs. This phenomenon has been suggested in rabbit aorta where it was observed that V and diltiazem were able to antagonize the effect of norepinephine-stimulated Ca transport in cholesterol-enriched but not control samples (Bialecki and Tulenko, 1989). Since different classes of CCBs appear to bind to different sites on Ca channel proteins, it may be speculated that cholesterol feeding alters the lipid composition and fluidity of the brush border membrane (BBM) and thereby affects the binding site for V differently from that of N.

The passive component of intestinal glucose uptake was differentially affected by N or V but only in animals fed a low cholesterol diet (LCD) (Tables 3-7, 3-8). In this case, it appears cholesterol feeding may "negate" the effects of CCB-induced alterations in uptake. The mechanism for the effect of CCBs on Pd^* are unknown, however, it is well documented that Ca is an important factor contributing to the integrity of tight junctional strands or complexes in the intestine (Donowitz and Madara, 1984). Thus, it might be speculated that chronic feeding of CCBs alters tight junctions in the intestine and that cholesterol feeding might prevent these CCB-associated alterations.

Several mechanisms of intestinal adaptation to various stimuli have been described including changes in the effective resistance of the unstirred water layer (UWL) or the lipid composition of the BBM (Keelan 1985a, 1985b; Brasitus et al, 1985; Thomson et al, 1984b, 1990). For example, the effective resistance of the UWL decreases in cholesterol fed animals which would be associated with a higher uptake of lipids (Thomson et al, 1987; Thomson 1982a). In the present study the effective resistance of the UWL was not assessed. However, we did observe a reduced uptake of most lipids in high cholesterol diet (HCD) plus V and no alterations in HCD plus N (Tables 2-7, 2-8). Furthermore, a change in UWL with N or V would be expected to affect the Km^* and Pd^* for hexoses in a similar direction, and this did not occur. In addition, changes in UWL would not influence the value of Jmax which

was observed in this study. Thus, it is unlikely that alterations in UWL resistance played a major role in the mechanism of the effects of N or V on lipid or hexose uptake in LCD or HCD.

In vitro, CCBs have been associated with the de novo synthesis of LDL receptors suggesting their potential for synthetic activity of membrane bound proteins (Paoletti et al, 1988). The induction of intestinal glucose transporters has been proposed as a mechanism for the enhanced Jmax observed in response to high carbohydrate diets fed to mice (Diamond and Karasov, 1984). The movement of lipids through the BBM is believed to be mainly a passive process although a carrier-mediated component has been suggested for fatty acids and possibly cholesterol (Stremmel, 1985, 1988). We did not assess the effects of N or V on BBM lipid composition, fatty acid binding proteins or glucose/galactose transporter(s). If CCBs do indeed stimulate the synthesis of glucose carriers, this might account for the lack of effect of N or V on glucose uptake in the acute studies (Tables 3-5, 3-6) where tissue exposure to these agents was limited to 6 or 36 minutes. However, decreased Jmax for glucose and galactose in the LCD plus V and HCD plus V group, respectively, do not support this potential mechanism.

The alterations in nutrient transport cannot be explained on the basis of variations in the animals' body weight gain, or food intake. In addition, there were no variations in mucosal surface area between drug administered versus control groups (Tables 2-4, 3-4), in spite of a significant increase in villus height in both HCD plus N and HCD plus V animals. Two dimensional measurements (villus height and width) of mucosal surface area do not necessarily provide reliable assessments of surface area. For example, in this study the villus density (number of villi/mm^2 serosa) decreased in HCD plus N and HCD plus V, countering the increase in villus height, so that mucosal surface area was unchanged.

A lack of correlation between mucosal surface area and nutrient transport function has been reported in other studies (Keelan et al, 1985a, 1985c). The functional surface area for uptake across the villus may not be the same for all nutrients (Chang et al, 1987; Cheeseman, 1986; Maenz and Cheeseman, 1986; Winne, 1978). Thus, changes in total villus surface area may not necessarily be associated with changes in the functional surface area used for nutrient uptake, and the lack of a change in villus surface area in an experimental setting does not necessarily signify the lack of change of the functional villus surface area.

Polyamines (putrescine, spermidine and spermine) and their rate-regulating enzyme ornithine decarboxylase (ODC) may be important in cell growth, replication, and differentiation (Pegg and McCann, 1982). In an osteogenic sarcoma cell line (UMR 106-01) V dose-dependently reduced basal and stimulated ODC activity (Langdon, 1984). The author suggested a role for Ca in basal ODC regulation, and a Ca, cAMP-dependent mediation of stimulated ODC activity. It is unknown what the effects of chronic CCB feeding on intestinal ODC activity might be. It has been observed that ODC levels are higher in villus than crypt cells, thus implying polyamine involvement in mature or differentiating intestinal cells (Baylin et al, 1978; Sepulveda et al, 1982). Thus, it might be speculated that altered ODC levels would affect the functional portion of the villus with resulting effects on nutrient uptake. If cell migration or turnover is affected by polyamines, then reduced ODC levels possibly achieved by feeding N or V might allow the enterocytes remaining on the villus to mature and to acquire greater transport potential. Static measurements of mucosal surface area do not indicate which portion of the villus and which particular functional component may be affected, nor do they provide information regarding cell proliferation, differentiation or migration rates. Thus, it is possible that feeding CCBs resulted in a change in the dynamic morphology of the intestine and thereby influenced lipid uptake. Furthermore, a more mature population of cells or altered distribution of transporting

enterocytes would be expected to be associated with an enhanced Jmax for glucose and galactose. Alterations in the distribution of transporting cells and "recruitment" of carriers to the lower portion of the villus have been suggested as a possible adaptive mechanism to experimental stimuli such as diabetes or radiation (Chang et al, 1987; Fedorak, 1990). However, speculations of the effects of CCBs on the dynamic morphology of the intestine await direct testing.

The Km* of the carrier for hexoses was affected by the chronic feeding of CCBs, reflecting an apparent alteration in the properties of the existing carrier(s) in addition to a change in their number. It is well known that the lipid composition of the membrane determines the physicochemical properties of the membrane and may have significant effects on the function of membrane-bound proteins (Yeagle, 1989; Clandinin et al, 1985). The lipid composition and fluidity of BBM has a significant effect on the kinetic properties of glucose transport in rat and rabbit vesicles (Brasitus and Schacter, 1982; Meddings et al, 1990). It is possible that CCBs affect lipid metabolizing enzymes such as fatty acid desaturase and acylation, deacylation enzymes or the cholesterol and phospholipid metabolizing enzymes, thereby resulting in altered membrane permeability and transport. A Ca, calmodulin dependent kinase has been suggested to play a role in the short-term regulation (via phosphorylation and concomitant inactivation) of HMG-CoA reductase, the rate limiting enzyme for cholesterol synthesis (Beg et al, 1987a, 1987b). Reduced Ca flux might upregulate the expressed activity of HMG-CoA reductase by promoting the dephosphorylation of the enzyme. However, if a Ca-dependent step was affected by the presence of CCBs in the enterocyte, it would be anticipated that both classes of drugs would affect intracellular synthesis of cholesterol and thereby would have directionally similar effects on BBM content of cholesterol.

Possible Ca involvement in acylation and deacylation enzymes in the enterocyte has not been studied (Berg et al, 1988). However, Ca may play a direct role in modulating membrane lipids by binding to anionic sites of the membrane bilayer. However, in rat BBM intraluminal Ca decreased the fluidity by alterations in membrane sphingomyelin content by unknown, presumably indirect mechanisms (Dudeja et al, 1987). Ca mediated alterations in lipid composition have also been observed in hepatocytes (Livingstone and Schacter, 1980).

Although it is tempting to speculate that CCBs might influence some of these metabolic processes in the enterocyte it would be anticipated that any Ca-mediated step would be qualitatively affected in a similar manner by both N or V. Thus, it would be expected that similar changes in Km"s would be observed for N or V in addition to directionally similar changes in lipid uptake; this was not the case in the present study.

In cultured macrophages, CCBs inhibit cholesterol esterification (Daugherty, 1987). The mechanism for this action has not been defined, but is apparently independent of the Ca-blockade effect and could involve a direct effect on the ACAT reaction in this tissue. Furthermore, not all classes of CCBs exhibited this inhibitory effect. It is unknown whether a similar effect might apply to the intestine, but clearly alterations in the activity of ACAT also influence the intestinal uptake of cholesterol. It is interesting to speculate that an upregulated state of ACAT following cholesterol feeding, as well as possible differential effects of CCBs on this enzyme, could account for some of the variable effects noted between CCBs and diet effects.

CCBs affect short circuit current (Isc) measured across ileal tissue with V having a quantitatively greater effect than other classes of CCBs (Homaidan et al, 1989). This represents the net effect of Ca channel blockade on the flux of anions and cations from the mucosa to the serosa and from the serosa to the mucosa. In the presence of CCBs equally enhanced mucosal to serosal movement of sodium and chloride exceed serosal to mucosal

movement of these ions. Residual fluxes (likely bicarbonate ions) and Isc are decreased (Donowitz and Asarkof, 1982). In the present study, the CCB-induced dose-dependent reduction of basal Isc was not observed in rabbit jejunum. This lack of effect could be related to differing electrolyte transport mechanisms between jejunum and ileum. Specifically, in rabbit jejunum there does not appear to be either NaCl symport or Cl/OH-antiport (Gunther and Wright, 1983). Since ion flux studies were not done in our study, it is not possible to comment on the direction or specific ions contributing to the Isc.

Glucose-dependent sodium fluxes are unaffected after short term exposure to CCBs in rabbit ileal tissue implying no effect of CCBs in sodium-dependent glucose transport. The results of the present acute exposure jejunal uptake studies support this observation. However, in chronically fed animals and in our Isc studies, it is apparent that glucose transport is significantly altered by the CCBs (Tables 3-7, 3-8; Figures 3-1, 3-2).

In in vitro nonintestinal cells, reductions in intracellular Ca induce alterations in sodium permeability; these effects are associated with enhanced activity of the sodium pump (Na^+/K^+-ATPase) (Hagane et al, 1989; Knudsen and Johansen, 1989). Whether Ca is involved in the physiological regulation of the sodium pump and whether chronically fed CCBs exert an influence on Na^+/K^+-ATPase in the intestinal basolateral membrane (BLM) is unknown. There appear to be at least two isoforms of this ATPase in the intestine (Fedorak, 1990) which could be differentially affected by cholesterol feeding and could be influenced differently by various classes of CCBs. Effects on sodium gradients may influence the uptake of nutrients. Removal of sodium from the bulk phase reduces the uptake of fatty acids (Thomson, 1982b; Stremmel, 1988) but it is unknown what an enhanced mucosal to serosal flux of sodium might have on lipid uptake. However, the variable effect of V on the uptake of glucose, galactose and fatty acid suggests that a general affect on sodium gradients is unlikely the sole mechanism mediating the observed changes in nutrient transport.

Glucose stimulated Isc responses were suppressed by the presence of V, N or N plus V in the serosal bathing solution (Figures 3-1, 3-2). This presumably reflects diminished sodium-dependent mucosal to serosal movement of the hexose which is unexpected in view of the enhanced mucosal to serosal movement of sodium reported in the presence of these drugs in ileum (Hubel and Callanan, 1980; Donowitz and Asarkof, 1982). Furthermore, the suppressed Isc response was observed in LCD but not HCD. However, the control HCD Isc response to glucose was lower than the LCD control values. Thus, since glucose Isc response was already suppressed in HCD versus LCD this may have accounted for the lack of further depression by CCBs. As discussed, the feeding of cholesterol may alter tissue properties in a manner that influences the interaction between CCBs and Ca channels. Furthermore, the fluidity and physicochemical properties of the membrane may be altered in HCD resulting in altered transport as described earlier. It is also possible that cholesterol modifies the properties of the tight junctions and thus the general permeability of the tissue. For example, the ionic conductance in the LCD control tissue was greater than the conductance in the HCD group. It is unlikely that these results were due to toxic effects or tissue death as theophylline significantly increased Isc in all tissues.

The results of our acute exposure study did not show any effect of increasing doses of mucosal N or V on in vitro glucose uptake. Based on these results in the acute study, it is unlikely that mucosal effect (i.e. modification of glucose carrier(s)) in the BBM is occurring after short term exposure to CCBs. However, the putative suppressed glucose transport in the Isc studies may be due to reduced activity of Ca-dependent potassium channels (CDPCs) proposed to exist in enterocytes (Brown and Sepulveda, 1985; Montero et al, 1990). Increased potassium permeability of the basolateral membrane may be coordinated with enhanced sodium entry across the apical membranae and it has been suggested that CDCPs may mediate this linkage. If potassium permeability plays a role in providing the driving

force for sodium-coupled transport across the cell it is possible that CCBs could influence the movement of glucose by affecting the activity of the CDPCs.

The implied CCB suppression of glucose transport in the Isc study differs from the results of our acute and chronic exposure in vitro uptake studies. The time-course of drug exposure may be a factor. In the acute exposure nutrient uptake studies the tissues were exposed to CCBs for 6 and 36 minutes, whereas in the Isc studies, CCBs were present in the medium for 72 minutes before the glucose-stimulated measurements were determined. Furthermore, as indicated above, the mucosal versus serosal exposure to CCBs may have significance. Further Isc studies with mucosal addition of the CCBs need to be conducted to determine if the site of CCB exposure has an influence in the jejunum. Finally, although the ionic fluxes imply that mucosal to serosal movement of glucose may be reduced by CCBs, the effects may not be of significant magnitude to translate to alterations on in vitro or physiological uptake.

It is not unexpected that the functional effects of the chronic study would differ fro the acute exposure studies. In acute experiments of drug exposure, the drug is directly exposed to either the mucosal or serosal surface of the tissue being studied. However, in a chronic administration study of CCBs, several factors need to be considered. In this study, the CCBs are administered orally and thus present to the luminal side of the enterocyte. Thus, the absorption of the agents may be variable and influenced by the luminal contents. In view of the lipophilic nature of these agents it is likely that their absorption will be enhanced in the presence of a high lipid diet. We attempted to avoid a possible differential effect in drug absorption between HCD and LCD by providing the drug at noon, when food intake by this species is generally low. However, variable absorption between drugs and between diets cannot be strictly ruled out.

Another consideration with the luminal presentation of the drug is that Ca channels are present predominantly in the BLM of the ileum (Donowitz, 1983). If this is the case in the jejunum, then the pharmacological agents must cross the enterocyte before exerting their blockade effect on the BLM. Although the presence of luminal Ca channels has been implied in jejunum and colon (Markowitz et al, 1985; Donowitz et al, 1985) this is uncertain. In rat jejunum intraluminal perfusions including 0.4 mM V stimulates Na and H_20 absorption. Thus, it is possible that the observed effects are not exclusively due to the Ca channel blocking effects but also luminal and other unidentified processes occurring during transport across the intestine. This does not make the results of this study less significant, as the majority of these drugs are taken orally. In terms of hemodynamics, the administration of oral versus injected CCBs exert qualitatively similar effects. In order to further delineate possible mechanisms of the results obtained in these chronic feeding studies, the use of intraperitoneal injections of these agents would be of interest (see Chapter V - Directions for Future Research).

The physiological significance of the findings in this study remain to be determined. Our initial interest in the possible effects of CCBs on lipid uptake was stimulated by the observation of cholesterol lowering effects (both serum and aortic) in animals fed N simultaneously with HCD (Senaratne et al, 1991). This cholesterol lowering effect of N was clearly not due to a decline in intestinal uptake of lipids (Table 2-7). While V reduced the uptake of lipids in HCD (Table 2-8), it did not reduce levels of cholesterol in serum (Table 2-3). In contrast, N reduces serum cholesterol levels while enhancing uptake. Thus it is likely that the effect of N on cholesterol concentration lies beyond the intestine, and points out that at least in the rabbit, reducing serum cholesterol levels may in fact be associated with increased intestinal uptake of cholesterol. This study does not rule out the possibility that CCBs alter lipoprotein synthesis and/or exit from the enterocyte.

Alterations to both the passive and active transport component contribute to the net effect of CCBs on intestinal hexose uptake. Passive glucose uptake may exceed rates of active transport at luminal concentrations in the physiological range after a meal (Pappenheimer, 1990). In the present study feeding LCD plus V reduced Jmax by 17.6% but increased Pd* by 29.4% versus LCD controls. If passive flux is 50% of the total glucose uptake after a meal (Pappenheimer, 1987) it may be speculated that V feeding results in a slight (6%) increased in net glucose uptake. N feeding enhanced Jmax by 29.5% and reduced Pd* by 12.2%, based on the above assumption, the net effect might be an 8.75% increase in uptake. In HCD, Pd* was not affected but Jmax increased with N or V feeding. Thus, although speculative, it is possible that CCBs differentially affect individual kinetic parameters but their effects on net glucose uptake may be directionally similar. Oral V has been reported to reduce glucose tolerance to a glucose load in normal but not diabetic volunteers (Ferlito et al, 1982). Other studies have reported no effect of CCBs on glucose tolerance (McTavish and Sorken, 1989). In the present study, the animals fed the CCBs had normal serum glucose levels in spite of altered capacities for intestinal glucose transport.

In conclusion, the results of this study suggest that the chronic administration of CCBs has the potential to influence the jejunal uptake of active and passively absorbed nutrients. The altered uptake likely occurs due to an intestinal adaptive response as the acute mucosal presence of these agents does not influence in vitro nutrient uptake. The direction of the altered uptake is influenced by the class of the CCB as well as the cholesterol content of the diet.

In addition, acute serosal exposure to CCBs does not influence basal ionic fluxes in the jejunum but does reduce glucose and theophylline-stimulated ionic movement. This effect is also influenced by the cholesterol content of the diet. The mechanisms and

physiological significance of CCB-induced alterations in nutrient and electrolyte transport are undetermined.

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CHAPTER V

DIRECTIONS FOR FUTURE RESEARCH

The present studies demonstrate that chronic feeding of nisoldipine (N) or verapamil (V) alters the in vitro jejunal uptake of lipids and hexoses in rabbits fed low cholesterol diet (LCD) or high cholesterol diet (HCD). The modification in nutrient uptake likely reflects an intestinal adaptive response that is differentially influenced by the class of calcium channel blocker (CCBs) as well as the cholesterol content of the diet. Future research should be directed towards testing the following hypotheses to: A) further delineate the specific site of intestinal adaptation and interaction with CCBs and B) determine the mechanisms mediating altered transport function.

5.1) SITES OF CCB INTERACTION WITH THE INTESTINE

HYPOTHESIS: Calcium (Ca) channels are present in the basolateral membrane (BLM) of rabbit jejunum.

It has been reported that in rabbit ileum, Ca channels and CCB binding sites exist in the BLM and not the brush border membrane (BBM) (Donowitz and Asarkof, 1982). The presence of luminal CCB binding sites has been suggested in rat colon (Donowitz et al, 1985) and jejunum (Markowitz et al, 1985), but it is unclear if this is also the case in rabbit jejunum. It would therefore be of interest to determine the effects of chronic N or V feeding in vesicles of isolated BBM and BLM. Furthermore, radioligand binding studies as done in rabbit ileal membrane preparations (Homaidan et al, 1989) would provide additional information regarding potential Ca channels and binding sites in the jejunum. In both vesicle uptake and radioligand binding studies it would be necessary to include animals fed LCD and

HCD to determine if the cholesterol content of the diet exerts a further influence. Ideally, to further define the functional component affected by feeding N or V, cells from different sections along the crypt-villus axis might be isolated from treated animals (e.g. crypt, mid-villus and villus tip) (Meddings et al, 1990) and vesicle transport studies could be conducted in the membranes from each fraction.

It is unknown if the observed effects of CCBs on nutrient transport are due solely to their Ca channel blocking activity or if they may be attributed to other unknown processes resulting from direct luminal drug exposure and transport across the intestine. In terms of hemodynamics, the administration of oral versus injected CCBs exert qualitatively similar effects (McTavish and Sorkin, 1989). A study of the effects of intraperitoneal injections of N or V on nutrient transport in both LCD and HCD animals would provide important information regarding direct luminal versus Ca channel blockade activity.

In the short circuit current (Isc) studies, only the serosal side of the jejunum was exposed to the CCBs. Therefore, studies with mucosal exposure to CCBs should be conducted to determine effects on Isc and provide further information regarding the site of intestinal Ca channels (see Hypothesis 2D). Only two classes of CCBs were studied in these experiments. In order to fully define the nature of the interaction of the various classes of CCBs as well as the differential effects of these agents, various combinations of all three classes of CCBs should be studied, including phenylalkylamines (Type I), dihydropyridines (Type II) and benzodiazapines (Type III) (see section 1.9.1). Possible mucosal and serosal additions of these drugs might include the combination of Type I plus Type III, Type II plus Type III, and Types I, II and III.

5.2) MECHANISMS OF ADAPTATION

HYPOTHESIS A: Intestinal BBM composition is altered by the chronic feeding of CCBs.

The lipid composition of intestinal BBM may be influenced by dietary manipulations, aging, diabetes, exposure to abdominal irradiation and ethanol feeding (Keelan et al, 1985a, 1985b, 1985c; Brasitus et al, 1985; Clandinin et al, 1985). It is well known that the physicochemical properties of the membrane and consequently, membrane fluidity influence the function of biological and enzymatic processes including nutrient transport (Brasitus and Schacter, 1982; Meddings et al, 1990). Furthermore, a potential role for Ca as a messenger in affecting membrane lipid composition has been suggested (Dudeja et al, 1987).

Thus, it is important to determine if feeding CCBs alters BBM lipid composition and if so, if the effects differ between classes of CCBs with or without LCD or HCD. The effects of feeding a HCD of 2.8% on intestinal lipid composition are unknown. Thus, this study would provide valuable information from both control and drug fed groups. In particular, it would be important to determine membrane total cholesterol and phospholipid (PL) content, the ratio of these, the composition of the PL subclasses, and the fatty acid composition of each PL subclasses.

If altered membrane composition was observed it would be ideal to measure dynamic and static components of membrane fluidity as previously described (Chautan et al, 1990; Meddings et al, 1990).

HYPOTHESIS B: Chronic feeding of CCBs alters the activity of membrane-lipid metabolizing enzymes.

Alterations in lipid metabolizing enzymes, particularly HMG-CoA reductase, PL metabolizing, acylation-deacylation and desaturases, are likely to influence the composition and biological function of the enterocyte membrane (Brasitus and Schacter, 1982; Garg et al, 1988; Dudeja et al, 1987). Ca may play a role in the short term phosphorylation-

dephosphorylation regulation of HMG-CoA reductase. Ca has also been implicated as a potential regulatory factor in the activity of sphingomyelinase and sphingomyelin synthase. It would be important to determine if these and other enterocyte lipid-metabolizing enzymes are influenced by the chronic administration of CCBs in LCD or HCD particularly if membrane lipid composition is altered by these agents (Hypothesis 2B). Enzyme assays of interest that are currently validated and available in the present laboratory include: phosphatidylethanolamine methyltransferase (PEMPT), phosphocholine transferase, and several desaturase enzymes.

In nonintestinal cells, CCBs may influence the activity of acyl cholesterol acyl transferase (ACAT) (Daugherty et al, 1987). It is probable that altering enterocyte ACAT activity might affect intestinal lipid uptake; thus an assay of this enzyme would also provide useful information regarding potential mechanisms for altered nutrient uptake in animals fed LCD or HCD with chronic CCBs.

HYPOTHESIS C: Polyamine activity and effects are influenced by chronic feeding of CCBs.

It is well known that polyamines are associated with cell growth and differentiation. In nonintestinal cultured cells a role for Ca has been suggested in the induction of basal ornithine decarboxylase (ODC) activity. Furthermore, V dose-dependently reduces basal and stimulated activity of this rate-limiting enzyme for polyamine synthesis (Langdon, 1984; Van Leeuwen, 1988).

The effects of chronic CCB feeding (plus LCD or HCD) on the production of polyamines could be determined directly by assaying intestinal concentrations of putrescine, spermidine and spermine as previously described (Hosomi et al, 1987; Langdon, 1984; Van Leeuwen, 1988). Measurements of ODC activity and use of the specific inhibitor of this enzyme (difluoromethyl ornithine (DFMO)) may also be used to characterize effects on

polyamine metabolism. Furthermore, if would be of interest to determine if CCBs modify the induction of ODC or polyamines in a physiological setting (e.g. after feeding or post-resection).

It would also be of interest to determine if the putative effects of polyamine including cell growth and proliferation are altered by chronic feeding of CCBs. Autoradiographic studies using tritiated thymidine to label cells entering mitosis and serial studies at set intervals after labelling would provide information of rates of cell migration. Johnson (1987) suggests that these types of studies should be combined with measurements of actual mitosis (DNA, RNA) to distinguish between uptake of label and actual incorporation into DNA. In the absence of changes in mucosal surface area (as reported in the present study) alterations in these dynamic components of intestinal morphology may represent the adaptive mechanisms accounting for CCB-induced changes in nutrient transport.

HYPOTHESIS D: CCBs alter intestinal ion fluxes in the jejunum.

It is well documented that the acute serosal presence of CCBs influence ileal ionic transport presumably enhancing mucosal to serosal movement of sodium and possibly reducing serosal to mucosal movement of residual (likely bicarbonate) ions (Donowitz and Asarkof, 1982; Homaidan et al, 1989). As illustrated in the present study, these effects may not occur in the jejunum. However, since radiolabelled undirectional fluxes of individual ions were not determined in these experiments it is not possible to delineate the contribution of various ions to the net Isc. Thus, in order to conclusively define the effects of acute CCBs on jejunal ion transport, this type of study needs to be conducted.

As indicated earlier, it cannot be assumed that Ca channels in the jejunum are located exclusively in the BLM of the jejunum. Therefore it is important to include mucosal exposure to CCBs in the proposed ionic flux studies.

Furthermore, there are no reports of Isc studies or ionic flux studies in animals chronically fed CCBs plus LCD or HCD. Studies in the chronically exposed animals are important because altered in vitro nutrient uptake was observed in the 3 week fed model of the present study and not the acutely exposed animals.

HYPOTHESIS E: CCBs influence Ca activated potassium (K) channels in the intestine.

It has been reported that Ca activated K channels (calcium dependent potassium channels [CDPCs]) are present in the BLM of rabbit and chicken enterocytes (Brown and Sepulveda, 1985; Montero et al, 1990). During active nutrient transport these channels may have an important role in regulating K permeability and energizing the transport processes. In the present study and others CCBs presumably reduce glucose transport (Montero et al, 1990). In order to determine if the effect is mediated via the putative CDPCs channels a study of the effects of CCBs on substrate induced K efflux, combined with Isc or ion flux studies using known inhibitors of Ca-dependent K permeability (either apamine, a peptide neurotoxin from bee venom), quinidine or barium should be conducted (Brown and Sepulveda, 1985). In isolated enterocytes concentrations of $5x10^{-7}$M apamine, 1 mM quinidine and 5 mM barium respectively, inhibit the K efflux induced by active transport. If Na-dependent nutrient transport is energized by Ca-activated K channels it would be anticipated that these agents would reduce nutrient transport.

HYPOTHESIS F: CCBs influence the function of tight junctions in the intestine.

The chronic administration of N or V resulted in altered uptake of passively absorbed L-glucose thus implicating a CCB-induced modification in the properties of the intestinal tight junctions. While Ca has been identified as an important factor in determining the structure of the tight junctions (Pappenheimer, 1990), it is unknown what influence chronic CCB feeding may have on these structures. Thus, future studies should be developed to further investigate this potentially important effect. Since intestinal conductivity (ie

resistance) is primarily determined by the properties of the tight junctions, Ussing chamber experiments would be a valuable tool in conducting these studies.

5.3) FURTHER REFINEMENT OF PRESENT STUDIES

A) Influence of lipid:taurodeoxycholic acid (TDC) ratio.

In the present studies the directional effects of N feeding on cholesterol uptake were influenced by the ratio of TDC to cholesterol in the experimental solution. It has been demonstrated in this study, and others that with an increasing ratio of TDC:cholesterol the in vivo uptake of lipids declines, likely due to reduced partitioning of cholesterol or fatty acid from the micellar to monomeric solution (Thomson and Dietschy, 1981; Thomson et al, 1983).

It is unclear why N feeding would decrease cholesterol uptake in LCD when 20 mM TDC was used to solubilize the lipid but enhance uptake when 10 mM TDC was used. Furthermore, this variability was observed in N but not V fed LCD and HCD groups.

Since all of the fatty acids studied were solubilized in 20 mM TDC it is unknown if a similar effect is observed in association with fatty acids. Thus, a future study using several varied ratios of TDC:lipid should be conducted to determine if this is a consistent phenomenon associated with N feeding. Furthermore, the enhanced uptake associated with a lower TDC:lipid ratio may be a more effective experimental setting to demonstrate the effects of CCB feeding, particularly for N.

B) Further study to differentiate intestinal lipid uptake differences in serum cholesterol-hypo- versus hyperresponders

There may be a great deal of inter-animal variation in the hypercholesterolemic response of animals fed a high cholesterol diet (see section 1.5.6). The mechanism accounting for the variability in response has not yet been determined. It is unknown if the

animals that fail to develop significant hypercholesterolemia have reduced intestinal uptake of lipids. Since altered transport capacities may significantly influence the interpretation of the results in the present study, it is important in future experiments to determine if there is variability in the intestinal function, composition and lipid-metabolizing enzymes between animals that exhibit different serum cholesterol responses.

C) Further study to determine alternate mechanisms for the hypocholesterolemic effect of the CCB N.

The present study provides evidence that the intestinal capacity for intestinal in vitro uptake of cholesterol is not altered by CCBs. Therefore, the cholesterol-lowering effect of N is not due to a decline in the intestinal uptake of lipids. The uptake of palmitic acid was not determined in the chronic feeding studies; in view of the association between high intake of this fatty acid and elevated serum LDL cholesterol levels (Bonanome and Grundy, 1988) it would be of interest to determine if CCBs affect its intestinal uptake. However, it is likely that the effect of N on cholesterol concentration lies beyond the intestine or at least beyond the point of mucosal entry. The cholesterol concentration in serum may be regulated by several mechanisms in addition to absorption from the gut including hepatic synthesis, secretion of lipoproteins and peripheral catabolism of lipoprotein species (Walldius, 1983). It would be useful information to determine which specific lipoprotein species in serum are influenced by the chronic feeding of N (i.e. LDL, VLDL, HDL).

General effects may also be determined by assaying the various lipoproteins and lipids present in the intestinal lymph and bile secretions and the effects of CCBs on the lipid composition of these fluids.

More in depth studies would need to be conducted to determine the effect of CCBs on hepatic synthesis enzymes (e.g. HMG-CoA reductase), catabolic enzymes (e.g. LCAT) and on LDL receptor and nonreceptor clearance of serum cholesterol.

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CITIZENSHIP: Canadian

POST-SECONDARY EDUCATION:

B.Sc. - 1982, Acadia University, Wolfville, Nova Scotia, Canada

R.D. - 1983, Health Sciences Centre, Winnipeg, Manitoba, Canada

M.Sc. - 1990, University of Alberta, Edmonton, Alberta, Canada

HONOURS AND AWARDS:

Honours achievement certificates 1975-1977

8th Canadian Hussaurs Association Bursary - 1978

Ruxby-Reid Gormley Scholarship - 1980

Sara J. Manning Bursary for Home Economics - 1981

Marion McColl Prize in Home Economics - 1982

Valedictorian - School of Home Economics, Acadia University - 1982

Carol Page Memorial Award - proficiency in Dietetic Internship - 1983

RELATED WORK EXPERIENCE:

Therapeutic/Education Dietitian, Selkirk Mental Health Centre, Manitoba 1983-1986

Clinical Dietitian - Cardiology, University of Alberta Hospitals, Edmonton, Alberta - 1987

Clinical/Teaching Dietitian II, University of Alberta Hospitals, Edmonton, Alberta - 1987

PUBLICATIONS:

Johnston E, Hyson D. Weight gain and related characteristics of pregnant Nova Scotian women. J. Can. Diet. Assn. 46:2-6, 1985

Hyson D, Monkhouse P, Mumme S. Cardiac transplantation: The Dietitian's role (abstr). Can. Diet. Assn., June 1987